

STRATEGIES FOR MANAGEMENT OF TRANSITION-CURVE USING RUMINE
NON-NITROGEN ENDS, SHORT DRY PERIOD AND DIETS TO IMPROVE THEIR
PERFORMANCE DURING LACTATION

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By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2002

To my mother Sarah Gilkey and my father Albert Gilkey
for their endless patience and support working toward
this goal and throughout my life. Without their sacrifice,
this may never have come about.

ACKNOWLEDGMENTS

I wish to express sincere gratitude to my advisor, Dr. R. Herbert Hood. I especially wish to thank him for his advice, kind help, support in solving problems and for providing the opportunity to begin a new career many years ago.

I also would like to thank my advisory committee members, Drs. R. C. Buckman, M. S. Hall, R. E. Sherman and F. A. Searles for their suggestions and support regarding my progress and dissertations and for taking time to read it.

My deepest gratitude is expressed to our lab chemist, Miss M. Joyce Hayes, for her help, friendship and knowledge. This study could not have been completed without her. I also appreciate collaboration of the three cores and the members of the Heavy Research Unit (HRLU). I thank the department of Animal Sciences for its financial support. I also would like to thank Pam Wilson for helping me complete various analyses.

I would like to express my thanks to my sisters, Anne and Cherie, and brothers to Joe, James/Hank and Daniel Tyson, who always encouraged me to seek the degree.

I would like to express my thanks to Maile who gave me the motivation to go on from one day to another. Thanks go to all my friends, especially Aydin Ozturkdoglu, Daniel Meier-Petersen, Sabina Tahir, Thomas Boehm, Whitney Labrecq and all those people who have emotionally supported me during my studies.

Finally I would like to extend my thanks to ALLAH for a faith that has anchored me through things and seasons of my life.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic Hormone
ADF	And Dehydrat Filter
AI	Artificial Insemination
BCS	Body Condition Score
BWT	Bornwe Somatotropic
BW	Body Weight
CCE	Cholesterol
CMD	Caring Month
CP	Crate Protein
CUF	Concentrate Balancing Factor
CS	Chemical Somatomedinotropin
CUO	Clean-up Day
d	Day
DA	Displacement of Hormone
DCAD	Dietary Cation Anion Difference
DET	Dehydration Treatment
DM	Dry in Milk
DM	Dry Matter Intake
DMY	Dry Matter Dry Period Treatment
EB	Energy Balance
ECF	Exhaled Carbonate
FCM	For Concentrated Milk
FCO	Forced Dry
GHG	Growth Hormone Releasing Hormone
h	Hour
IGF-I	Insulin-like Growth Factor I
INS	Insulin
INE	Iron Enzyme
VLND	Very Low Density Lipoprotein
MIN	Milk Use Nitrogen
MY	Milk Yield
NDP	Neutral Dehydrat Filter
NDS	Negative Energy Balance
NDA	Net Exhaled Fatty Acid
NE	Net Energy Index
P ₁	Proteinase
PC	Protein Catabolism
PEPCK	Phosphoenol Pyruvate Carboxy Kinase
POF ₁	Proteinase P ₁
PL	Plasma Lactogen

PBL	Peptide
PTH	Parathyroid Hormone
hST	Recombinant Human Somatostatin
RDP	Rarely Degradable Protein
RR	Ratios- <i>in vivo</i>
RLP	Rarely Undegradable Protein
RCC	Ratios-Cell Count
RCM	Ratios-Cell Count
RGA	Ratios
RH	See Hematology (Domain 2)
SB	Somatomax
ST	Somatomax
STAT	Signal Transduction and Activation of Transcription
T ₁	Transferrin
T ₂	Thyroxine
TD	Triglycerides
TdR	Total Tumor Ratio
TET	Treatment
Wk	Week
Yr	Year

*Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree Doctor of Philosophy*

**STRATEGIES FOR MANAGEMENT OF TRANSITION COWS USING BOVINE
SOMATOTROPIN (ST): SHORT DRY PERIOD AND COWS TO IMPROVE THEIR
PERFORMANCE DURING LACTATION**

By

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August 2002

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Major Department: Animal Sciences**

Our objectives for this experiment were to evaluate the effects of a low dose of ST preparation and progesterone on BCS, DFM, DFM:ST, KPIII, BCS and some metabolites in plasma. It was carried out during two consecutive years. Holstein cows were assigned randomly to one of two groups (Control (C)-100, treated (T)-100 cows). Recently inseminated or ST were from 30 d before expected calving through 42 d postpartum (C: 10 to 1.0 to 10.0 mg/MTM, PMSLACTO). During year 1, ST, KPIII, BCS, NEFA and glucose were measured in plasma of 100 cows. During year 2, BCS and DFM of 110 Holstein cows were evaluated, but cows were not bled. Milk yields through 100 d were analyzed during both years. Progesterone: MT increased mean concentrations of ST and BCS but not glucose, NEFA, or KPIII. Progesterone treatment increased concentrations of ST and NEFA, but not BCS, KPIII, or glucose. Mean BCS did not differ prepartum or postpartum, but MT were maintained better DFM prepartum. The MT was greater for

hST rejected cows during the first 60 d of lactation, but not during the first 100 d. The second experiment included 50 Holstein cows to evaluate DMG, MY, metabolic hormones, metabolites and Ca. The 3x3x2 factorial arrangement of treatments included dry period (0 d dry, 30 d-dry+DGP, and 60 d dry+L), propionate and propionate hST (0 or 1 mg/d) and propionate source as calcium salt. Propionate hST increased concentrations of KT, RFP-1, DGL and glucose. Propionate hST increased concentrations of KT and RFP-1 in plasma but not glucose or NEFA. Glucose MY was observed for hST across through the first 21 wk but hST did not differ due to dry period. Injections of DGP at dry-off did not improve MY. Cows with shorter dry period experienced lower BW and DCS as well as 60 d dry cows. No effect of propionate dose on DMG, MY, or blood measures including Ca was detected. Data indicated that hST treatments and short dry period can be used as management tools to improve the performance of lactating cows and their subsequent milk production.

CHAPTER 1 GENERAL INTRODUCTION

Dairy cows undergo gestation and parturition on a regular cycle during the year they live on the producing farm. The change from the nonlactating stage to the lactating stage is very demanding on the cow. Early lactation and peak lactation are the two periods during the lactation cycle when nutrient demands are rapidly increasing and become greatest for cows.

Another important period during the lactation cycle is that from 21 d prepartum to 21 d postpartum which has been termed the "transition period" (Drinking, 1999). Cows undergo many physiological changes during the transition period. Pregnant cows usually show reduced DM during late pregnancy and the early postpartum period. It has been shown that live weight decreased approximately 30% during the final days before calving (Nisbet et al., 1952). Importantly, many metabolic diseases such as fatty liver, ketosis, milk fever, rumen acidosis, and displaced abomasum (DA) are most likely to arise during the late pregnancy and/or early lactation periods (Dell and Hume, 1953).

Lactating dairy cows show peak milk yield between 4 and 12 wk postpartum. During early lactation, cows are deficient in energy and other nutrients (Whitaker et al., 1999), largely because peak DM is not reached until 18 to 19 wk postpartum (Peters et al., 1952). High milk secretion associated with high demand for glucose and protein generally gives rise to increased mobilization of body fat and protein reserves. However, cows mobilize relatively more energy than they do amino acids to support milk

production because of energy and nutrient deficiencies. This often leads to the health problems seen during early lactation. If the additional energy and protein needed to meet the cow's requirements are not supplied by the diet, then they must be met by tissue mobilization and that may be a major cause of the development of metabolic diseases during the transition period (Drackley, 1999).

Goals for the transition cow are to decrease excessive mobilization of fatty acids from adipose tissue storage and transport to liver and to minimize the depletion of glycogen stores from the liver. Most important, if feed intake can be maintained or even increased during the transition period and throughout early lactation, when milk yield is also increasing, the cow better maintain the energy balance needed to keep cows from turning into metabolic disturbances that have bad consequences. Rapid and large rate of increase in BMB during early lactation is essential to provide energy and nutrients to support a rapid increase in milk yield. Knowing that feed intake is a key point on the opportunity to derive status to keep a high, and to use or develop technologies or feeding strategies to bring about desired effects especially during the last 3 wk of dry period.

One of the major products of biotechnology is recombinant bovine somatotropin (rBST or simply bST). Use of bST during lactation has resulted in exceptional increases in milk production by dairy cows (Bauman, 1999). The development of recombinant biotechnology enabled the development of rBST which provided an abundant and affordable source of ST for research and, subsequently, for commercial application. Initially, availability of bST allowed researchers to conduct the first studies to better evaluate its effects. These studies showed that bST increased milk production by 10 to 15 % in dairy cows (Bauman, 1992). Bovine somatotropin has been used consistently for

more than 10 yr in North America and about 8 yr in the UK to improve milk yield of cows in commercial dairy herds.

Use of hMT during the preparation period may offer a means to cause positive and beneficial effects on metabolism. This is because of the known positive effects of hMT on feed intake during lactation and on health of the expected cow (Eppard et al., 1993).

Recent researches also has the ability to partition nutrients both acquired and those mobilized from liver and muscle to the mammary gland during lactation. These effects if started during the preparation period, may allow the close-up cow to better make the transition to high demand for energy and nutrient intake and the active function of the liver and other organs that provide the substrates for many important metabolic pathways. It clearly has been shown that low doses of hMT have positive effects on metabolism, hormones and milk production when expected. HMT preparation through hMT (Bancroft et al., 1992).

Somatotropin is a primary hormonal regulator during pregnancy and lactation (Bancroft and Vernon, 1993); it regulates processing of nutrients (carbohydrates, lipids, proteins, and minerals), and plays an important role in the coordination of various organs and tissues (Bancroft, 1992). To support its synthesis, metabolism of other tissues is maintained to provide the necessary precursors. Somatotropin also has a major role in the regulation of IGF concentrations in the circulation, besides like growth factor I is a local mediator of the mammary epithelial growth and development where it stimulates the cellular activity of mammary gland (Phillips et al., 1990). Increase in IGF concentrations in peripheral plasma is associated with sustained exposure of IT and the increase parallels that of hMT during the lactation period.

Some historical period trials that were performed to evaluate the effects of injecting a full dose of bST (500 mg/1.4 d) (the previous often had negative effects on injected cows. Despite large increases in milk production during the few few weeks after parturition, feed intake did not increase immediately after bST treatment and resulted in severe negative energy balance resulting eventual loss of BCS and subsequent reproductive problems (Hindrichs et al. 2009). However, it has been hypothesized that use of a low dose of bST injected during the transition period would improve a cow's overall performance, especially during lactation, without the negative effects seen in earlier trials when greater doses were used. Janssens et al. (1998) concluded that when bST (3 and 6 mg/d) was injected into cows during the last 48 d before parturition, DM intake is to be about 2 kg/d greater after parturition. Butler et al. (1994) reported that postpartum treatment with bST resulted in higher mean DM intake after several weeks of injection and that the increase was dose dependent. Garcia (1998) reported that cows injected with 3 mg bST/d during both the prepartum and postpartum periods had greater DM than untreated controls or those cows injected with 3 mg bST/d only during the prepartum or only during the postpartum periods. In addition, reports by Kertz et al. (1991) and Garcia (1998) indicated that cows in the untreated control group had the greatest loss in BCS and those cows did not begin recovery of body condition until 8 wk of lactation.

Maintenance of lactation can be described as maintaining the number of secretory mammary cells and their synthetic activity during a defined time period. Change in the amount of milk produced during a defined time period can be used to measure maintenance of lactation. Along with mammary gland related factors such as removal of the milk, other factors such as environmental management, nutrition, genetics

and milking frequency affect lactation persistency, a measure of maintenance of lactation in dairy cows, after peak milk production is reached. gradual involution of mammary tissue occurs during regressing lactation in spite of the frequent removal of milk. Hence, a decrease in daily milk volume follows as the number of functional differentiated epithelial cells in the mammary gland decreases. Part of the reduction in milk yield also may be due to a reduction in the milk secretion rate of each of the functional cells that do remain. (Maphum, 1987)

On the other hand, dairy cows require a nonlactating dry period between successive lactations. Mammary involution in this period is characterized by a decrease in the total number of alveoli per lobule, a decrease in the total number of alveoli and lobular volumes, a decrease in the number of cells per alveolus, and an increase in the length of alveolar cells (Schwartz, 1975). Involution in the dry period is an essential process for the mammary gland so that recovery of body reserves can occur to support subsequent lactation and so that lactation also can be sustained at a high level. A period of 45 to 60 d generally has been recommended for the dry period (Smith and Tordella, 1982). According to Coppock et al. (1979) less than a 45-d nonlactating period decreases milk yield in the subsequent lactation, whereas greater than a 60-d nonlactating period increases feed costs without associated return and can cause a decrease in the lifetime production of the cow. However, cows do not produce milk during the dry period. One method to increase lactation milk production by individual cows would be by manipulating the length of the dry period. If the dry period could be shortened and if the rate that the mammary tissue involution could be accelerated, then perhaps one could decrease these "unproductive" days, and yet still achieve maximal milk yield during the

most lactations. In this way total milk yield could be increased but the same number of cows would be milked.

Mammary involution is a fairly rapid process that occurs after cessation of milking. Regression of mammary secretory tissue accompanies dramatic changes in composition of secretions during the transition from lactation to involution. As indicated previously, dairy cows require a nonlactating dry period before calving to achieve maximal milk production during the next lactation (Cappuccini et al., 1974; Bradley, 1989; Elam and Woodward, 1983; Kitchell and Henderson, 1972). Adequate proliferation and differentiation of mammary secretory epithelial cells during the nonlactating period are essential for optimal secretory function in the subsequent lactation and the duration of the nonlactating interval is critical negatively to milk production (Elam and Henderson, 1983).

Plasma and its inactive components, plasminogen, two of several significant proteases in bovine milk (Oguri, 1977) have been implicated in the destructive process during the gradual involution that occurs in lactating udders. Plasmin is an extracellular serum protease which is formed by cleavage of a peptide bond in the single polypeptide chain of the inactive precursor plasminogen (Henderson et al., 1980). Plasmin degrades milk casein mainly in its inactive form. Stage of lactation affects plasmin milk level; lactation associated with higher concentrations (Priden et al., 1984). They proposed that the increased plasmin activity in milk during late lactation may be involved in subsequent mammary gland involution (Priden et al., 1980).

It has been suggested that antigens administered at cessation of milk removal could accelerate the resolution of mammary tissue (Allen et al., 1986) by accelerating the

activation of plasminogen (Alba et al., 1977). They reported that concentrations of plasminogen, plasmin, and rennin cells in secretions were increased earlier in Holstein cows exposed with 11 mg of estradiol-17 β on each of the 4-d that preceded final milk removal than in control cows exposed with 4 mL of oilseed. In addition, the ratio of plasminogen to plasmin in secretions obtained earlier for treated cows than for control cows (Alba et al., 1977). As a result, absorption of ingested nitrogen at final milk removal suggested the normal mammary involution process, accelerated active involution and still left enough time for the mammary regrowth phase. These results suggested that the shorter dry period could be incorporated into a dry period management scheme without any adverse effects on the milk production during the subsequent lactation.

To be an effective strategy to improve milk production, shortening the dry period must be coupled with a good nutritional management program to allow cows to maintain body condition and good health after parturition. An important advantage of a superior feeding program during shortened dry periods would be the ability to maintain good rumen function when cows were fed during the shortened dry period that were formulated from concentrates similar to those used to formulate the lactation diet. This would encourage maintenance of a population of rumen microbes well suited to the lactation diet for postpartum. Also, cows would have the ability to maintain good postpartum milk production whether cows or calves were fed, then they would begin involution in lactation without energy and metabolic stress.

Total cow numbers in Florida are about 120,000 and average dry period length is 70-d. If it can be shown that cows with a 30-33 d dry period would produce just as much

milk during the next lactation, as those with 60-70 d dry period and with no negative effects of short dry period on health or subsequent milk production, then an opportunity exists for dairy producers to earn extra money. This extra income would arise from the extended number of days as milk during the current lactation. For example, at an 18-20 kg of milk/d could be sold as a week of extra days as milk that would otherwise be lost if the cows went dry for the full 60-d. Of course, in order to adopt this practice, cows should be producing enough milk at the 60 d dry period mark and have a good BCS (minimum 3.25), and they should be given sufficient nutrition to support their needs during the next 30 d of lactation.

The surface of the rumen mucosa is characterized by rumen papillae, which can be defined as regions of absorption. Their distribution, size and number are closely related to feeding habits, forage availability and digestibility. The typical features of rumen papillae are genetically fixed but may vary considerably under different feeding conditions, resulting in acute and usually temporary or seasonal adaptations (Delouis et al., 1983). For example, increasing proportion of forage and propionate results that increase rumen blood flow also stimulates mucosal mucus. This results in vascular dilation and epithelial cell proliferation. Thus, there are increases in number and size of papillae within the rumen. Changes in the number and development of rumen papillae in response to nutritional changes require an adaptation period of 2 to 3 wk (Delouis et al., 1983).

Microorganisms in the rumen depend on the rumenist to provide the physiological conditions necessary for their existence. In turn, these microorganisms are essential for digestion and fermentation of large amounts of fibrous feeds that the

nutrient consumers, but otherwise could not use efficiently. Thus, by providing a suitable and consistent environment for these microorganisms, the rumen is able to use the end-products of fermentation to meet its own nutritional needs. Comparison of rumen microorganisms shows that there is a high level of variation among cows. The large diversity in the types of microbes found in the rumen is a reflection, in some extent, on the diet. Growth of microorganisms and efficient fermentation of food by microorganisms depends on a constant and suitable environment (Van Soest, 1982). Changes in feed and feed management (as well as rumen pH) cause a shift in microorganisms in the rumen and also a decrease in the efficiency of the fermentation and absorptive efficiency of the fermentation. Changing the diet of the animal provides a period of transition in the rumen microbial population which causes the proportions of the different species in the rumen to shift until a new balance is established, one which best accommodates the dietary changes. This is referred to as adaptation of the microbial population. Adaptation typically takes several days to weeks to occur (Jenkins et al., 1981; Yokoyama and Johnson, 1985).

The current standard 181 study period allows nutritional management of dry cows to be organized in two different phases: the off (FOD) and close-up dry (CUD) periods. During these periods, diets given to animals vary due to the metabolic differences of cows during these short time periods. Diet changes from lactation diet to the off diet, from the off diet to close-up dry diet, and from close-up dry diet to lactation diet forces transition in the rumen to adapt these diets during a short time period. It also is likely that feed intake is changing and feedlot to new approach eating. These changes in diet offered probably further decrease the feed intake and likely limit the rumen in feed intake.

immediately after parturition. This is a concern because early lactation is when greater feed intake and more efficient forages most increases of ingested feed is desired. If length of the dry period can be decreased to ~30 d, then a better feeding program can be developed with these formulated feed components similar to those that are used to formulate the lactation diet. This will encourage maintenance of a better mass, selected populations, better udder development, and greater progeny survival, reproduction. Therefore it should allow greater and more rapid increase in feed intake, should maintain efficient fermentation and digestions, and should allow better protection to metabolic demands during early lactation.

The major objectives of this research were to evaluate the use of hST during the transition period and to evaluate dry period length. Thus, two studies were conducted at the Dairy Research Unit (DRU) of the University of Florida. The main objectives of the first study were to evaluate the effects of hST on feed intake, BCS and BW pre- and postpartum, to evaluate the overall yield of milk during lactation, and determine any adverse or positive effects on the health of the animal. The main objectives of the second study were to evaluate dry period length (30 vs. 35 d), the use of hST to evaluate speed of dry-off, the types of progeny metabolic data (positive or negative), and supplemental treatment of hST during the transition period by measuring DMI, BW, BCS, milk production, and udder health during subsequent lactation.

CHAPTER 1 USE OF MGT IN MANAGEMENT OF THE TRANSITION DAIRY COW TO INCREASE FEED EFFICIENCY, IMPROVE MILK YIELDS AND DECREASE HEALTH PROBLEMS

Introduction

During the early 20th century nutritional discoveries allowed better understanding of the basic biology of growth and development. Biotechnology followed the advances in applied nutrition science and had an important impact on agriculture as well. One of the major products of biotechnology is recombinant bovine somatotropin (bST or rST) which resulted in an exceptional increase in milk production by dairy cows when injected during ongoing lactation. For example, the theoretical annual gain in milk yield with routine recombinant technology can be estimated as follows (40% AI and semen mixing and AI and embryo transfer can be as much as 100, 110 and 120 kg, respectively (Boman, 1996). However, improvement in milk yield by administration of bST can result in 2000 kg of additional milk yield per lactation (Boman et al., 1993). This increase would be equal to that normally achieved by AI and genetic selection over a 10-20 yr period (Boman, 1996).

As early as the 1930s, growth promoting effects of ST were characterized. Davis and Sampson (1937) showed that crude extracts of bovine pituitary increased the growth rate of rats. In 1933, two Russian scientists, Kharin and Krasov, performed the first bST research using 400 lactating dairy cows. These researchers found that

improving cows with evidence of the potential of slaughtered cows resulted in increased milk production. Research continued during and after World War II as scientists sought an effective means of increasing food production. However, the amount of ST from milk plasma was small that this source would not be practical for improving milk production (Young, 1947).

As indicated, the arrival of modern technology enabled the development of recombinant bovine somatotrophin (rBST) which provided an enhanced source of ST for research and potentially for commercial application. This allowed researchers to conduct many studies using recombinant rBST. From these studies it was concluded that exogenous ST could increase milk production of dairy cows at least 26 to 29% (Rasmussen, 1982).

Objective of current research was to evaluate the effects of propionate and propylthiouracil treatment of cows with hST on milk yield, BCS and BW and to evaluate some important metabolites hormones during the treatment period. This study was conducted at the Dairy Research Unit (DRU) of the University of Florida and used Holstein cows.

Literature Review

Regulation of Somatotropin Secretion

Secretion of ST from the anterior pituitary gland is controlled by a complex neuroendocrine system that triggers neural effector stimuli. The results in stimulating rapid release of ST secretion through growth hormone releasing hormone (GHRH) and somatostatin (SR) of the hypothalamus, respectively. Decreased secretion of the

neuroendocrine hypothalamic nucleus, where secretion of GnRH occurs, results in a marked increase in plasma concentrations of ST. The mammalian testis and ventral-lateral hypothalamus are the only regions that are capable of this response. Stimulation of the paraventricular neuroendocrine area resulted in a decrease in blood concentrations of ST (Marta, 1972).

Negative feedback control of the pituitary is exerted at the pituitary level by GnRH. Besides the growth factor 1 also acts on the hypothalamus to stimulate secretion of GnRH, whereas β -adrenergic stimulation inhibits GnRH secretion (Marta, 1972). Neurotrophins, a neuroendocrine neurotransmitter, regulates the release of GnRH and inhibits the hypothalamic portal blood. In vivo, inhibition of neurotrophins synthesis or blockade of $\alpha 2$ -adrenergic receptors reduces concentrations of ST in plasma and decreases spontaneous pulses of ST (Mellon, 1987). On the other hand, activation of $\alpha 2$ -adrenergic receptors stimulates secretion of ST (Tory and Martin, 1986).

Somatostatin, a secreted from somatostatin cells located in the anterior pituitary gland. Somatostatin impulses from the paraffinity terminate in the hypothalamus, stimulating neuroendocrine nuclei in this area. These neuroendocrine nuclei secrete GnRH or inhibin in the capillary loops of the median eminence. Capillaries converge into the portal trunks of the neural stalk, pass in the anterior pituitary gland, and break up into various sinusoids where they terminate or inhibit the secretion of ST (Johlin et al., 1989). Thus, a variety of neuroendocrine factors as neurotrophins, dopamin, and somatostatin play a role in the neuroendocrine regulation of ST secretion. Growth hormone releasing factor and inhibin are somatostatin cell neuropeptides-cytokine transducers.

system. Glucocorticoids having releasing/secretory roles on secretory receptors that activate adenylylate cyclase via interaction with the regulatory coupling G-protein. Activation of adenylylate cyclase converts ATP into cAMP which activates cAMP-dependent protein kinase and this results in secretion of ST. Somatostatin acts via its inhibitory receptor and inhibits the activation of adenylylate cyclase. In rat neurohypophysis *in vivo*, low concentrations of Ca caused reduced secretion of ST (Shanbali et al., 1988). In addition to this finding, GHRH enhanced extra-cellular Ca levels when SR reduced the level of Ca in neurohypophyse cells (Riad, 1988). Thus, several phospholipids and Ca also were explained to be involved with neurohypophyse cell secretion of ST.

Evidence suggests that neuronal mass plays a major role in determining circulating concentrations of ST. Secretion-related elevated concentrations of ST in pigs (Arrese et al., 1978), sheep (Driver and Forbes, 1983), and humans (Phemister, 1983). Underhill even described higher concentrations of ST in plasma than well-fed lambs (Riad et al., 1979). Thus, it is not surprising that non-restricted fatty acids (OEFAs), glucose, leptin, and neurotrophin Y also appeared to influence release of ST (Smith et al., 1994). The direct inhibition of ST release from the anterior pituitary gland by NEFA is speculated to complete a feedback loop because ST is known to stimulate lipid metabolism (Jorda et al., 1986; Smith et al., 1994). However, a decrease in plasma NEFA caused a rise in plasma concentrations of ST (Johnson, 1984).

Glucose is an important regulator of ST secretion. Hypoglycemia stimulated secretion of ST in humans (Frederick et al., 1973), whereas acute administration of glucose inhibited secretion of ST (Quarles et al., 1978). In contrast, INS inhibited

hyperpolarization or intracellular glutamate released potentiated ST secretion in rats through stimulation of ES release from the hypothalamus (Terasawa and Mutoh, 1990). Lysine appeared to stimulate ST secretion by elevating GABA and ES release from the hypothalamus (Dawe et al., 1997).

Sexual Pattern of Secretion

The secretion pattern of ST is probably different between species studied. In the adult rat, secretion of ST was synchronous with ST secretory peaks that occurred at 3-4 h intervals (Terasawa et al., 1990). In male rats, ST secretion occurred in discrete pulses with low interpeak levels, whereas female rats showed less pulsatility and higher interpeak levels (Clark and Sakuma, 1982). Women have higher overall concentrations of ST with a higher pulse amplitude and baseline, but the frequency of pulses were the same as in males (Jalilov et al., 1995). In normal secretion of ST in rats followed an episodic pattern rather than being synchronous. Episodic pulses of ST were seen throughout the 24 h cycle. There appeared to be considerable variation in the secretion pattern of ST among individual rats and no association of time of day or time of day was observed (Dawe et al., 1997). Rats exhibited greater amplitude of ST episodes that occur with no significant differences in baseline values (Dawe et al., 1997). Basal and pulsatile release of ST were regulated differently by age and sexual experience. Average concentration of ST in plasma was lower in female rats than in females at 3 mo of age and basal secretion of ST was correlated negatively with circulating estradiol, whereas it was correlated positively with circulating testosterone (Jalilov et al., 1995).

Somatostatin Gene

The ST gene is a part of a large gene family including ST₁, perlecan (PRL-3), and the placental lactogen (PL) which they are known to share some common sequences (CB, Simer et al., 1986). The latter are thought to have arisen as a result of gene duplications. The ST gene has five exons and four introns, spanning approximately 3.6 to 3.8 kilobase pairs in most mammalian species including rabbit. In chicken and rodents, most the gene is 3.3 and 4.5 kilobase pairs, respectively, due to the larger intron sizes (Toskins et al., 1983; Argenteau et al., 1983). In primates, unlike in most mammals, there are multiple ST genes that include ST-H and ST-V. The ST-H gene is expressed in somatostatic cells of the anterior pituitary, whereas the ST-V gene is expressed in placental tissues (Tuggle and Toskins, 1986).

Structure of Bovine Somatostatin

Bovine somatostatin is composed of 140 or 146 amino acids and has a molecular mass of approximately 12,000 daltons (Anderson, 1986). The amino acid sequence of bST which gives it its three-dimensional shape, differs by about 10% from that of human ST (Cort and Fournier, 1976). The amino acid sequence number 127 in the sequence can be either leucine or valine. In addition, ST containing only 140 amino acids has a phenylalanine at the N8, whereas other than leucine that is found in the 146 amino acid form of ST. These differences occur because of a different cleavage of the signal peptide and thus, four different variants of bST are produced naturally (Wood, 1984).

Because monomergous has four subdomains it helices packaged close together which gives the protein a relatively rigid appearance with a high thermal stability (Figure 1.10). A bend in a helix, smaller than of 36.2 degrees is caused by aspartate and aspartate 89 is proline. This proline consists of 14 amino acid α -conserved throughout ST of different species (Clarkson et al., 1991). Because monomergous consists mostly of a hydrophobic core. In the helical region there are 40 hydrophobic side chains. Over 90% of all hydrophobic chains in the helices are buried inside due to either the packaging of the helices or loops (Clarkson et al., 1991). Disulfide bridges are known to contribute significantly to the stability of the molecular structure of many proteins (Ravett et al., 1989). The connection assembly the two disulfide bonds of ST between the helices and loops plays the key role in stabilizing the protein for discrimination and mechanical interactions.

The disulfide bridges are formed between cysteine residues with both connecting a loop to a helix. The first disulfide bridge connects the hooking motif of one of the long loops (aspartate and aspartate 31) with helix 4 (aspartate and aspartate 144) which pulls the long loop into the surface of the bundle (Oliver et al., 1989). The second disulfide bridge breaks the small loop of the C-terminal segment (aspartate and aspartate 144) to the 4th helix (aspartate and aspartate 180) which forces the C-terminal segment to bend toward the helix bundle (Ravett et al., 1989). The loops of the ST, which hold the cysteine residues for the disulfide bonds, consist of 32 hydrophobic side chains. Of the 32 side chains 17 are completely or partly buried either between the helix and loop or between the loops themselves (Clarkson et al., 1991). The structure of



Figure 2-4: Time dimensional steps of MEE (Cichocki et al., 1994)

BT is composed of four beta helix bundles. The first α helix is composed of 24 amino acids, the second is composed of 21 amino acids, the third is composed of 22 amino acids and the fourth has 30 amino acids (NishiMiyazaki et al., 1997). The rest of the protein is composed of two long loops with 46 and 24 amino acids, respectively and a short loop with 9 amino acids. The long loops are between helices 1 and 2 and between 3 and 4. The short loop is between helices 2 and 3 (Chakrali et al., 1994). Six amino acid residues are attached to the N-terminus of α helix number 1 and 1 amino acid are attached to the C-terminus of alpha helix number 4.

Glucocorticoid Receptor

Glucocorticoid marks cellular response as a result of its specific membrane bound receptors. These receptors are the first of the class II cytokine receptors to be cloned (Liang et al., 1987). The cytokine receptor superfamily is composed of 15 members including PRL-receptor-like (2 through 7), erythropoietin, interleukin 36, and the leptin receptors. This family of receptors has common domains such as a single extracellular spanning domain, two parts of cytoplasm and a conserved tyrosine adjacent to the cytoplasm in the N-terminal module, clusters of tyrosine protein kinase activity, and a proline rich region in the cytoplasmic domain (Bazan, 1993). These proline-rich regions are critical in signaling because they bind the Janus Kinase (JAKs) the major mediators of this class of cytokine receptor (Wilson et al., 1999).

The cytokine receptor superfamily has a three-domain organization including an extracellular ligand binding domain, a single transmembrane segment and an intracellular domain. The mature (52) receptor is 428 residues long, with 246 residues of

the extracellular domain and CXX-motifs of the intracellular domain. The extracellular region also contains 2 fibronectin type-III domains that are closely related to FRII and syndecan-like receptors (Zlotowski et al., 1995). Human ET receptor consists of 38 genes spanning 81 kb, with the receptor itself being coded by 9 exons that yield an mRNA of 6.3 kb (Zlotowski et al., 1995). Multiple-exon loci allow the regulation of receptor expression in different tissues in response to different stimuli (Adams, 1993).

The mechanisms by which ET regulates the transcription of genes required for body growth and its regulation are being delineated. It has been suggested that signal transducers and activators of transcription (STAT) are key contributors to ET signaling and to the mechanisms by which ET activates genes that lead to morphological actions (Figure 3.2). STAT1, 3, 5a and 5b are tightly regulated by ET-ET receptor and JAK2 interactions and participate in the regulation of many genes associated with growth and metabolic effects. Somatotropin uses two different sites to bind selectively to two different receptors. Transactivation of receptors by the hormone causes activation by bringing the extracellular domains into close proximity. This dimerization then activates the receptor associated JAK family of tyrosine kinases. JAK2 phosphorylates tyrosines within itself and the ET receptor. These tyrosines form binding sites for a number of signaling proteins, including members of the family of STATs which play very important roles in the regulation of gene transcription (Wells, 1996). STAT proteins are latent, are homologous domain 2 (SH2) containing cytoplasmic factors. Studies with ET receptors indicated that JAK2 can activate STAT 1, 3, 5a and 5b. Activated STAT proteins then yield hetero- or homodimers via an SH2 phosphorylated

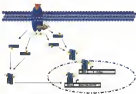


Figure 2.8. Signaling and regulation of the transcription factor by ST. Adapted from Harrington et al. (2000).

lysine interference which enters the nucleus of the cell, binds to DNA, and initiates transcription of target genes (Schaeffer and Garavito, 1993).

Response to Gonadotropin Implantation

Gonadotropin is a major regulator of growth in mammals. In the boar GT regulates the expression of a wide range of proteins including hormone receptors and growth factors, secondary products, and enzymes such as cytochrome P450 (Norman et al., 1990). Gonadotropin also has a unique effect on stimulating mammary gland development (Goldman et al., 1990) and lactation (Rafter et al., 1992).

Typical MY response to hMT is an increase of at least 18-25%. However, the response can be much greater with better care and management of the sows (Bauman et al., 1985, NRC, 1994). After the first few days of hMT treatment, MY increases gradually and reaches a peak, and the increase is maintained with continuous treatment of hMT. Milk yield gradually returns to normal pre-treatment levels after cessation of treatment (Bauman et al., 1990).

Obtaining a milk yield response to hMT does not require special diets or different feed ingredients. Substantial milk yield response have been observed on diets ranging from percent to the more typical total mixed ration (TMR) diets. However, dry matter intake (DMI) increases on hMT treated sows after a few weeks of the supplementation and persists throughout the interval of hMT use. Thus, treated sows require adequate amounts of a balanced diet rather than a special diet (NRC, 1994).

Effect of IGT on the Mammary Gland

In early years of study on action of IGT it was concluded that a direct effect of IGT on mammary gland development was unlikely. Studies failed to detect an IGT receptor on the mammary epithelial cell of the cow (Gordon et al., 1984). Mammary ablation of IGT directly into mammary story of sheep did not increase milk production (McDermott et al., 1987). However, an indirect, a direct effect of IGT on mammary development and function was established (Pitt and Gordon, 1984; Kinsberg, 1987). Although most evidence suggested an indirect effect of IGT on mammary gland development in ruminants, it was found that ruminants also expressed mRNA for the IGT receptor in mammary gland (Khan et al., 1988). Furthermore, immunologic staining of IGT receptors in mammary tissue of pregnant and lactating cows also was reported (Pitt-Gibber et al., 2004). Gibber et al. (1997) observed a significant effect of IGT on mammary growth of pregnant females when it was administered through the test canal. However, as indicated, unilateral cross a total ablation of IGT into one half mammary gland of sheep did not increase milk yield performance over the unoperated half (McDermott et al., 1987). Furthermore, IGT administered through the test canal of lactating goats did not result in increased milk production response (Bjorseth and Knight, 1994). This latter response would be expected unless there was uptake of IGT across the apical membrane of the epithelial cells to uptake into the general circulation and subsequent action in mammary gland.

The acute rise in IGT response in hGT and rapid decline after discontinuing hGT suggest rapid uptake and cell proliferation being caused by IGT in the short-term.

Knight et al. (1984) observed that ST treatment did not affect the survival of mammary parvocytosis and brief lutealizing of mammary-apical cells in vivo during the first 6 wk of lactation. On the other hand, a large increase in parvocytosis release was observed in most lactating goats treated over a 22 wk period with exogenous ST (Knight et al., 1984). Other studies with cows and goats have established trends such as significant increases in lipoprotein due to MT in the mammary gland such as acetyl CoA carboxylase, acetyl CoA synthetase and fatty acid synthetase. Thus, MT directly and/or indirectly causes an increase in the rates of milk synthesis per cell and an improved maintenance of mammary cells (Barnes and Vernon, 1990; Barnes and Barnes 1993).

One of the mediators by which ST affects mammary gland function is via an indirect effect on mammary tissue by action of IGF-1 (Cobble, 1990). Concentrations and actions of IGF-1 likely are the most important link in tissue response when higher concentrations of ST occur either because it is expected or because of greater secretion than the natural primary. Secretorin plays a major role in regulating the concentrations of IGFs that act in the circulation. Concentrations of IGF-1 increase during MT treatment via both induced release of IGF-1 from a hepatic storage pool and because of greater biosynthesis of IGF-1. Biosynthesis is regulated by increased mRNA levels and mRNA stability (Shotton et al., 1994). Increase in IGF-1 concentration was associated with continued exposure of ST and the increase in MT was parallel to the increase in IGF-1. Decrease of ST supplementation produced a parallel decrease in both MT and blood concentrations of IGF-1 (Shotton et al., 1994).

Insulin-like growth factor I has both mitogenic and postmitotic actions in addition to its endocrine action (Johansen et al., 1992). At the cellular level, IGF-I locally stimulates amino acid transport, synthesis of RNA and DNA, and synthesis of cellular proteins (Phillips et al., 1992). Receptors for IGF-I were demonstrated on mammary tissue and IGF-I was a local mediator of mammary epithelial growth and development (Perry et al., 1984; Vago et al., 1991). Intralobular vascular cells, small blood vessels, and capillaries contain IGF-I receptors in the mammary gland (Silman et al., 1992). During ET treatments, IGF-II binds to the cytoplasm and the nucleus of epithelial cells. Insulin like growth factor-I stimulates the cellular activity of mammary gland, it increases synthesis of RNA and DNA, and the synthesis of cellular proteins. Therefore, it is a local mediator of mammary epithelial growth and development (Phillips et al., 1992). Interestingly, close arterial infusions of IGF-I into mammary gland of goats, but not systemic infusions of IGF-I, increased MT dramatically (Petersen et al., 1994). However, intramuscular injection to close arterial infusion of IGF-I was much less than that of systemic IGF-I treatments which suggests that the mechanism of action of IGF with regard to stimulatory effects on MT cannot be localized only to the known effects of IGF-I.

Effects of Somatotropins on Other Tissues

Somatotropin exerts many different effects on protein metabolism in both mammary and non-mammary tissues. Besides somatotropin increases milk yield with no change in milk composition, when there is energy deficiency, it provides milk yield without increase of an overall increase in blood supply to mammary gland (water

uptake) alters glucose metabolism (glucose synthesis in the mammary gland), enhances lipolysis and increases lipogenesis in the mammary gland (supply of lipid precursors), and also modulates protein metabolism (supply of amino acids). As a result, milk yield increases and the composition of milk does not change from normal (Bauman, 1990).

Increased MT response due to ST is due mainly to altered partitioning of resources to liver of mammary glands and to an increase in the synthetic capacity and/or longevity of the milk synthesizing cells (Bauman and Vernon, 1990).

Somatotropin is a primary locomotive regulator during pregnancy and lactation (Bauman and Currie, 1988). It regulates partitioning of nutrients (carbohydrates, lipids, proteins, and minerals) and plays an important role in the coordination of various organs and tissues (Bauman, 1990). To support milk synthesis, the metabolism of other tissues is stimulated to provide the necessary precursors.

Carbohydrate metabolism:

During early lactation, glucose is used almost exclusively by the mammary glands (mammary non-oxidative system and lactose), and MT is heavily dependent upon maternal glucose supply to the gland. If MT is to increase milk yield, then it must act in a way to direct more glucose to the mammary gland. This can occur through a variety of individual actions of IGF. First, hMT increases mammary blood flow so that more blood perfuses the mammary gland and increased uptake of glucose can occur (Serafini et al., 1983). A decreased ability of IGF to inhibit gluconeogenesis is observed following hMT treatment. Both *in vivo* (Coburn et al., 1985) and *in vitro* (Kagge et al., 1982) studies demonstrated that hepatic rates of gluconeogenesis were stimulated

during treatment of dairy cows with SST. Furthermore, ST decreased sensitivity of IRE receptors to IRE in the peripheral tissues and this resulted in decreased overall uptake of glucose in peripheral body tissues. This diminished the oxidation of glucose to CO_2 , and as a consequence more glucose was made available to the udder. Somatotropin also stimulated increased feed intake, and as a result, more propionate was produced in the rumen; rumen acid became available for gluconeogenesis (Bunce *et al.*, 1982). *In vivo* studies of liver tissue of ST hypersecretors showed a 50% increase in capacity to use propionate for gluconeogenesis (Krupp *et al.*, 1981).

Pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK) are potential rate-limiting enzymes for hepatic gluconeogenesis during the transition period (Cresswell *et al.*, 2000). It was speculated that exogenous ST would increase mRNA synthesis in the liver that coded for these enzymes (Sawyer, 1995). On the other hand, Paving *et al.* (2004) concluded that maintenance of milk production was not evaluated through increased liver gluconeogenesis in dairy HFDH. Somatotropin also increased lipid mobilization and more glycerol thus was available as a precursor for gluconeogenesis. The main goal of these various would be that higher blood glucose concentrations occurred and that glucose could be directed to the mammary glucose support lactation. In addition to increased glucose production in the liver, glucose usage by other body tissues decreased. Because glucose is used as the primary energy substrate and as a substrate for synthesizing milk constituents, energy needed by other peripheral body tissues would be derived from products of lipolysis or

metabolism of non-glucotogenic compounds arising from the rumen and lower digestive tract.

Lipid metabolism

Neuronal insulin plays a major role in the regulation of lipid metabolism. Insulin promotes SST growth to stimulate rumen both lipogenesis and lipolysis in adipose tissue with the net effect being related to energy balance (SR) (Boman, 1999). When cows are in negative EB, synthesis and deposition of lipids in adipose tissue are reduced by ST which, in turn, increases availability of nutrients and their utilization for milk production (Boman and Vernon, 1993). Insulin is an important homeostatic control in the regulation of lipid metabolism. Somatostatin reduces the ability of INS to stimulate lipogenesis in adipose tissue. Thus, ST reduces the action of INS, suppresses lipogenic enzyme activity, and reduces glucose uptake (Boman and Vernon, 1993). These coordinated changes in IGF system would support production of glucose from available precursors and conservation of glucose for mammary use by shifting peripheral tissues to utilization of other substrates available, at large part, due to coordinated actions of ST and INS.

When cows are in negative EB, ST stimulates lipolysis, it alters the sensitivity of adipose tissue to β -adrenergic agents (Boman and Vernon, 1993). Therefore, for cows that are in negative EB and are being treated with SST, increased lipid mobilization would be a major source of energy needed to support milk production (Dechow et al., 1993). Lipolysis is regulated by a signal transduction system that includes cAMP, stimulatory G proteins (G_s) and inhibitory G proteins (G_i).

Catecholamines act through the G_i system to stimulate lipolysis, whereas adenosine causes an antilipolytic effect via the G_o system. When adenosine binds to its receptor it stimulates G_o , which inactivates G_i , protein activation of adenylyl cyclase catalyzed by catecholamines. This leads to the inhibition of the lipolytic pathway that is stimulated by catecholamines. Bombesinogen affects lipolysis through an increase in response to catecholamines with no change in sensitivity. Epinephrine challenge following ST treatment dramatically increased NEFA concentrations in plasma. Interestingly, ST treatment resulted in modest changes in β and α_1 adrenergic receptor numbers (Kawano et al., 1992), the activity of G_i proteins was reduced significantly by ST treatment. As a result, it has been suggested that ST increased the ability of G_i to interact with adenylyl cyclase. This in turn would increase the effectiveness of G_i system stimulated by catecholamines (Barnes, 1993).

The events described above would dramatically increase milk fatness of lipids from the adipose tissue, and increase blood NEFA and glycerol. Thus, there would be greatly reduced fatty acid synthesis in the adipose and liver, less acetate and glycerol use in adipose tissue (Barnes et al., 1994). In, the net result would be a shift in the availability of these metabolites in the mammary gland where they can be used for synthesis of short and medium chain fatty acids that are themselves used for TG synthesis and milk production. Therefore, lipolysis also must be an important pathway to provide needed precursors in the early postpartum period by cows typically to supply the energy needed for milk production (Baldwin and Knapp, 1990; Barnes, 1993).

Acute acid metabolism

Insulinomimetic treatment increases milk protein synthesis in lactating cows via improved efficiency of amino acid utilization. A reduction in circulating urea nitrogen and a urinary nitrogen loss was reported following MCT treatment (Davis and Collier, 1985). During negative energy balance NT will spare protein use as a source of energy in tissue because it increases lipid mobilization and enhances glucose metabolism. Proteins that are mobilized from the muscles can be used in the liver, in the gut, and in the blood which will increase overall metabolism and efficiency of protein use. Amino acids mobilized here do support growth of some organs (liver, heart, digestive tract) rather than to support milk synthesis (Johnson and Anderson, 1985). Amino acids are mobilized to provide energy will be reduced such that the protein mobilized can be used for growth of specific tissues and milk protein synthesis. As indicated, NT causes an increase in blood urea which will make more nutrients and amino acids available to support increased milk yield, and that will leave the need for tissue mobilization.

Increased Blood Flow

It has been established that nutrient supply to the mammary gland is one of the major limitations for the activity of mammary cells and milk synthesis. A lactating mammary gland places a heavy demand on the animal to provide substrates for milk synthesis (Davis and Collier, 1985). With MCT exposure, along with the increases that occur in MIV, udder output also increases. Insulin like growth factor I has a role in the increased blood flow to the mammary gland that appears to be mediated by production

of milk yield (Peters et al., 1994). Somatotropin also increases conversion of T_4 to T_3 , specifically in the mammary gland and the increase in T_3 has a direct effect beyond because increases lipid metabolism and helps to mobilise the gluconeogenic response (Capuco et al., 1994). Thus, both increased milk secretion and local metabolism drive more of the blood circulation to mammary gland and this, in turn, supplies greater quantities of water and nutrients needed for milk synthesis.

Because of its positive effects on blood glucose, lipids and water and concentrations and blood circulation, hST increases milk yield without affecting the overall composition of the milk. In early lactation, amounts of long chain fatty acids available increases relative to short chain fatty acids and, generally, during negative energy balance, hST tends to increase milk fat and decrease milk protein. However, the effect is not great and milk protein does decline the effect disappears.

Transition Period

Transition from pregnancy to lactation is one of the most important challenges faced by dairy cows during a lactation cycle. The cows physiological status during the last 3 wk of the dry period through the first 3 wk of the subsequent lactation can have significant effects on the lactation and on reproductive performance (Bruckley, 1999). Following parturition, nutritional requirements of cows increases greatly due to the increased milk production. Dominant DMI gain is coming earlier over time the period immediately following calving and results in slower increase in milk yield. The DMI increased proportion is used almost totally for milk synthesis. This results in cows

undergoing a period of NFB because milk yield increases midway the month in mature adults (Bell, 1990).

Food consumption by the neonates now decreases by as much as 30% within a day or two before calving (Baron et al., 1992; Grossman, 1993). However, this decrease does not occur only during the final weeks preceding parturition. Pregnant dairy heifers showed reduced DMi from wk 76 of pregnancy (3.37% per wk) until 3 wk before calving (Ingerson et al., 1992). A similar decline was observed in both lactation and/or during the last 144 d of pregnancy (Ingerson et al., 1997) when they were fed a high energy diet (11.8 MJ of metabolizable energy/kg of DM) whereas the decline was less when they were fed low-energy diets (6.2 or 8.3 MJ of metabolizable energy/kg of DM).

During late pregnancy, fetal metabolic rate increases dramatically as a time when the demand on DMi also is increasing most rapidly. The fetal metabolic rate at this time is approximately 2 fold greater than that of the dam on a 10% bean hay-based diet (Farré, 1983). The source of carbon for oxidation in the growing fetus is mostly glucose and amino acids. Uterine uptake of glucose (Wagham et al., 1984) and/or acids (Sniffen et al., 1992), and acetate (Bell, 1990), as a source of maternal supply, is as high as 46–72 and 12%, respectively. As maternal energy-depleted states are especially susceptible to lypolysis/ketosis during late pregnancy (Ingerson et al., 1996) whereas glucose falls and high plasma concentrations of NEFA prepartum occur in lactating (Lambilliot et al., 1990).

Total energy requirements in the 1st half of pregnancy were 2.3 Mcal/kg ME, whereas the energy requirements increased to 26 Mcal/kg ME in some averaging 30 kg milk per day (Bell, 1992). Lactating dairy cows reach their peak MEY between 3 to 13 wk postpartum, and the lowest DMI occurs at calving. Peak DMI is not reached until 8 to 14 wk after parturition (Whitlock et al., 1995). DMI increases by approximately 1.5 to 2.5 kg/week during the first 3 wk of lactation (Ruvinsky et al., 1992). However, there is great variation among individual cows as to when they reach peak DMI. This time can be affected by pregnancy and postpartum stress, and degree of fitness or BCS (Brewer et al., 1998). In addition, DMI of periparturient cows is less than that of nonlactating cows (Kanev et al., 1994). There is great variation among peak DMI in cows. For example, peak DMI of some has been reported to be 2 and 11.1% more than DMI of the same cows at one week postpartum (Jones, 1992).

Delayed increases in DMI, with respect to increasing energy requirements, result in NEER at or soon after parturition. High producing cows show a net energy deficit of ~12 Mcal/d during their second week of lactation (Challinor, 1995). High-producing cows can mobilize 10 kg of body lipids and 15 to 20 kg of body protein to support the lactation (Whitlock et al., 1995a), whereas there is a need for more than 5 kg glucose per day for a cow producing 33 to 40 kg of milk daily during early lactation (Wheeler, 1988). Cows can mobilize 0.28 kg/body fat and 0.36 kg protein/d and the largest part of this mobilization (13% of total fat and 55% of total protein) takes place during the first week of lactation (Trenkner et al., 1987). As a result, the additional energy and

protein) need to be supplied to transition cows to support lactation depends the metabolism of development of metabolic diseases was to be revealed.

Metabolic Adaptations at the Transition Cows

During late pregnancy and continuing into early lactation, major changes in metabolism of the cow occur to cope with the increase in nutrient requirements for mammary metabolism. Responses for lactation are supported, in part, by an increase in DM and digestion, as described above. However, "understanding or overhauling changes in the metabolism of body tissues are necessary to support a physiological state" (Boman and Curran, 1988), in this case to support lactation, and it must accompany the increase in DM and digestion. These homeostatic changes include hormonal changes, increased hepatic metabolism, lipid and protein mobilization, and a reduction in the utilization of glucose and amino acids by lower priority organs (Reid, 1993). In this way the nutrient supply to mammary gland can be increased and enhanced.

Hormonal Changes

Concentrations of various hormones change as a relatively narrow time period around calving (Figure 1-1). Plasma concentrations of P_r peak around 4-280 ng/l protein (7-8 ng/ml). Thereafter, concentrations begin to decline to a 3-4 ng/ml, and on the day of calving concentrations of P_r are almost undetectable. By midlactation, nitrogen concentrations are from 28 μ g/ml, to 300 μ g/ml. Approximately 7 d before parturition, plasma concentrations of nitrogen decrease to around 2800 μ g/ml, whereas just before calving, the total nitrogen (free and conjugated) are about 4000-5000

pg/ml (Cline et al., 1977). Plasma cortisol concentration increases to 4-8 ng/ml 3 d before calving to peak around calving or the day after calving (15-30 ng/ml). The concentrations of cortisol and estrone decline to essentially basal levels within days after calving (Tucker, 1988). Around 24-36 h before calving PGE₂ concentrations begin to rise and they peak at calving. Plasma PRL increases rapidly the day before calving. Changes in plasma concentrations of PRL, estrone and P₄ are responsible, in part, for the increased synthesis of colostrum. Circulating concentrations of IGF usually increase before calving and remain elevated during early lactation, especially at high producing rates (Blaumen and Vernon, 1993). Even though concentrations of IGF in circulation remain high during the preparation period, concentrations decrease rapidly after calving. During the first few days after calving the basal concentrations of IGF-1 is negatively correlated with ET. Plasma glucose concentration increases during early lactation, but changes in the IGF-1 or glucose itself has more physiological importance because it is one of the factors determining the rate of lipolysis and lipogenesis (Sutton et al., 1984).

Lipogenic tissue

During early lactation a lot of energy is required for the changes that take place in adipose tissue. In mammals, the major site for tissue lipogenesis is adipose tissue. During early lactation fatty acid synthesis and esterification, plasma TG uptake by adipose tissue, lipoproteins, and the enzymes controlling these events are notably altered (McNamara and Hillers, 1986). There also is a reduction in the utilization of glucose, amino acids, glucose and TG by the adipose tissue. These events lead to higher entry of

glycerol and NEFA, from the adipose tissue into circulation and to a decrease in the rate of adipocyte

lipolysis. Inhibition of adipose lipolysis leads to many changes in the activity of a number of important enzymes of adipose tissue which are advantageous to milk production. Activity of lipoprotein lipase declines rapidly in adipose tissue and this leads to reduced uptake of fatty acids. Activity of the most important regulatory enzyme of lipogenesis, acetyl CoA carboxylase, declines dramatically during early lactation as well. The fall in the rate of esterification in adipocytes also is important for lipolysis because it means that less of the fatty acids that are released during hydrolysis of TG, are re-esterified (Varcoe et al., 1987). Although capacity for lipid synthesis decreases, the ability to hydrolyse adipose tissue derived TG increases rapidly. Increased activity of hormone sensitive lipase results in a further increase in lipolysis. As a consequence there are increased concentrations of NEFA and glycerol in the blood during early lactation.

Liver

Increased concentrations of NEFA in the blood before and at parturition results in increased uptake of NEFA by the liver, increased fatty acid esterification, and increased storage of triglycerides (TG) (Gronauer, 1995). Lower TG storage prior to or early during lactation because the rate of TG synthesis is positively associated with plasma concentrations of NEFA (Rea, 1986). Although esterification of NEFA to TG increases during early lactation, discharge of TG from liver in the form of very low density lipoproteins (VLDL) is very slow and limited in cases which likely causes fatty

liver before and up to calving, and during the early weeks of lactation (Cromartie 1980).

Some portion of NEFA taken up by the liver is oxidized to CO_2 and ketone bodies. Ketone body production increases when the ability of the liver to export fatty acids as lipoproteins is exceeded, and a glucose from (liver glycogen) at a time when concentrations of both glucose and FFA in blood are low (Lafont et al., 1981). Because peripheral utilization of ketone bodies is limited and there already is high entry of NEFA into liver, this may predispose animals to ketosis during early lactation.

Liver glutamyltransferase and glucose kinase increase greatly to support high demands by mammary gland after calving. Dietary glucose can only account for about 10 to 25% of glucose that is secreted in milk of a cow producing 40 kg milk daily. There is a marked increase in entry of gluconeogenic substrates such as propionate, succinate, glycerol and lactate both from the digestive tract and from metabolites of body tissues (Wenger 1984). The blood flow to the liver, weight of liver, and the activity per unit weight also increase during early lactation. The increase in liver weight, however, is proportional to that of increased protein synthesis (Kelly et al., 1981).

Muscle

During early lactation a loss of skeletal muscle protein has been reported for cattle (Blaxter et al., 1967) and sheep (Bryant and Smith, 1982). It was suggested that this was due primarily to the increased degradation of protein-rich milk or to a change in protein synthesis (Bryant and Smith, 1982). Amino acids in the peripheral circulation

that come from muscle can be used either for protein synthesis by the mammary gland or for gluconeogenesis in the liver.

Well-fed, high-producing cows can mobilize up to 20 kg of body protein without a health risk during the first 60 d of lactation (Whitelow et al., 1988). In underfed cows, even though the contribution of muscle protein can be one-half of the total protein utilized, potential mobilization cannot exceed more than 10% of body protein (Wilson et al., 1982).

Adapted mobilization of amino acids does not mean, however, that energy requirements of skeletal muscle are decreased. Although energy requirements of the muscle are the same, the source of energy utilized by the muscle shifts from glucose to fatty acids. Glucose uptake by muscle and the proportion of aerobic pyruvate dehydrogenase decreases during lactation (Niswender et al., 1987), whereas there is increased use of fatty acids and ketones for oxidative purposes by muscle during lactation (Potholakis and Lindsay, 1983).

Soils and minerals

Dairy cows have the highest calcium turnover per kilogram of BW during milk production (Grosser et al., 1980). During the last week of gestation, the fetus requires approximately 3 g Ca and 1.5 g P/d. At the onset of lactation, the daily excretion of Ca and P in milk is approximately 36 and 22 g, respectively (Lengemann, 1979). Up to 60 g Ca/d can be excreted in milk in the first 60 d of lactation suggesting that plasma Ca must be removed 25 times daily (Harris, 1984). Although voluntary absorption of Ca and its efficiency increased, this is not enough to supply Ca, but that

to the increased MY. Thus, animals must mobilize their bone Ca and other minerals to meet the requirements of the buffer hypothesis. This mobilization represents about 20% of minerals in developing early lactation (Mason et al., 1984). Failure to respond to low plasma Ca concentrations results in hypocalcemia (Jensen and Rasmussen, 1987).

General Comments

Important changes occur in metabolism of the dairy cow to support increased nutrient demands of the mammary gland during early lactation. These adaptations are coordinated by changes in the secretion rates of the minerals. Prolactin has an important role in the development of the mammary gland and IGF has an important role in coordinating the metabolic adaptations occurring in the body to support lactation. On the other hand, the release of IGF is reduced significantly as a consequence of the negative energy balance during early lactation. Although tissue response or responsiveness to IGF decreases, the response to catecholamines is enhanced. Thus, changes in both the responsiveness of tissues to hormones and overall circulating concentrations of hormones appear to have important roles in the metabolic adaptations seen during this critical time period during lactation and continuing throughout the lactation.

Materials and Methods

One hundred twenty-three multiparous Holstein cows from the University of Florida Dairy Research Unit (URU) herd were used in an experiment completed over a 2 yr period and the protocol approved by the Institutional Animal Care and Use Committee of the University of Florida. Cows were assigned randomly about 4 wk

prior to expected calving date. Age of the cows ranged from 3 to 6 yr and parity was between 1 and 5. Information on the animals, including parity and age, day and month of expected calving, day(s) dry, and milk yield during previous lactation were obtained from records of the DBU. Actual number of days that cows were sampled prepartum (25–104) differed from expected because of early or late calving.

During year one, plasma samples were collected from 81 Holstein cows [Control (C)=44 vs. treated (T)=47], and all cows calved between October 1998 and January 1999 at the DBU. During year two, effects of MT on BCS and BW of 111 Holstein cows (C=57 vs. T=54) were evaluated, but no blood samples were collected. These cows calved between October 1999 and March 2000. The BCS (1–5, thin to fat, Edmonson et al., 1989) and BW of cows were recorded (8–26–11–62 kg) before a m feeding test after a m milking. The BW and BCS of the cows at the time the trial started ranged from 304 to 870 kg, and 3.60 to 4.75, respectively.

Experimental Design

Cows were assigned randomly to one of two treatment groups. Treatment group 1 (48 cows) were controls and received no MT treatment, whereas those in group 2 (43 cows) received injections of 0.4 mL of MT (POSLACB) biweekly. The volume of POSLACB contained approximately 140.8 mg MT and provided about 10.2 mg MT/d.

MTT Description

A sterile, prolonged-release, injectable formulation of a recombinant DNA-derived bovine anemopoietin analogues (MTT, POSLACB, 500-mg mL⁻¹ in oil).

Monsanto, St. Louis, MO) was used for injections. Injections began approximately 4 wk (± 3 d) before expected calving dates. Regardless of time of last injection before calving, first postpartum injections were within 24 h of calving and thereafter injections were at 2-wk intervals. Last injection was at 43-d postpartum. Injections were subcutaneous in the post-scapular region or on either side of the subcostal flanks. Injections were administered after blood collection, but were prior to a.m. feeding or milking. No bST injections were given between 63-d postpartum through 300-d postpartum. All cows assigned to TRT 1 and 2 received a full dose of bST (500 mg/2wk) beginning at 100-d postpartum.

Measurements

Feeding program

Starting 4 wk before expected calving dates, cows were fed the close-up dry ration (Table 1). The CUD ration was formulated to be similar (~ 10 May 1995) [26] to decrease the postpartum risk of hypocalcaemia. After parturition, all cows were fed a total mixed ration (TMR) based on corn silage, whole cotton seeds (WCS) and grass concentrate (Table 2, 3). Clean fresh water was provided in water troughs and was available free-choice in the free stall barn where they were housed. Barns were equipped with fans and openers which helped cool cows when ambient temperature was above 25 °C.

Body condition scores and body weights

Body weights and BCS (1–5, that is fat, Edmonstone et al., 1985) of cows were recorded biweekly on the same day each week (Saturday) before a.m. feeding or

Table 2.1. Dry Matter Compositions and Chemical Compositions of CLD Rumen and Feed.

Ingredients	% DM	
	CPB	TMR
Core/Silage	37.12	24.32
Alfalfa Hay	—	8.34
Concentrated Molasses	—	3.97
Corn Silage	—	8.84
Haylage	33.24	14.29
Durum Wheat	1.44	9.88
Soybean Meal	1.44	3.89
White Chickpeas (PCC)	1.24	14.86
Mineral Mix	—	3.30
Springer Minerals	4.89	—
Barnyard Hay	39.38	—
Truss Minerals	5.39	—
Environ Phosphate	9.42	—
Chemical Compositions	Percentage ²	
DM	54.54	62.77
CP	16.28	17.67
Std CP ¹	24.88	33.41
ADF	23.94	31.33
NDF	27.24	37.14
EE ²	4.65	1.63
TDS	48.19	48.21
Wt. (dried kg)	1.32	1.68

¹ Analysis of components from NRC/BLA Forage Laboratory, Ithaca, NY. ² DM basis.² Percentage of the CP¹ plus values.

walking (11:00 to 12:00 h) during the second year. Measurements before the day-cows were assigned to trial and continued up to 150 d postpartum.

Blood collection, handling and storage

Blood samples were collected from the tail vein of all cows before the a.m. feeding or walking (07:00–09:00 h) during the first year. Cows were tied after shorting the tail without any other restraint. The order in which cows were sampled on a given day was random and differed from Yearling-day to Yearling-day. Cows were tied the day they were assigned to the trial and liberally during the prepartum period, the week of calving, and then monthly up to 60 d postpartum. For blood collection Vacutainer blood needles (1.29 cm, 20 gauge) and tubes containing sodium heparin were used (all in 100-mm blood collection tubes; Becton-Dickinson, Franklin, NJ). Blood samples were placed on ice immediately after collection and processed within 2 h.

All samples of blood were centrifuged at 1600 g for 10 min at 4°C for 20 min to the 60–80 refrigerated centrifuge (E-plane-mixing fraction H 605A rotor, IECall Instruments) in separate plasma. Plasma from each sample was aliquoted into two labeled 3 ml, polyethylene tubes (75x12 mm, capped, and frozen at -20°C until analysed. The plasma samples were used for analysis of IGF-1, IGF-1, and IGF-1 by specific radioimmunoassays (RIA) in radioimmunoassay-informative method of White (HPLC, White-Pure-Chemical Industries, Osaka, Japan) was used for the quantitative determination of IGF-1 in plasma as described by Johnson and Petras (1992). Sigma product No. 510 (Sigma Diagnostics, St. Louis, MO) was used for the quantitative

enzymatic determination of glucose in deproteinized plasma samples as described by Karim and Faridullah (1980).

Milking and milk collection

All cows were milked in a double 12 hump-horn milking parlor equipped with 24 DeLaval milking machines and a Galaxy 2000 milk recording system. An automatic cow identification system was used during the experiment, and individual milk yields were recorded at each daily milking from 14 different partitions to 4100 partitions. Cows were milked three times daily (04:30, 13:00, and 24:00 h). They were brought to the milking parlor holding pen before each milking and waited automatically by pulsing speakers placed on the floor beneath the covers (3 cycles, about 5 min). After milking was completed, cows were not clipped using unlabeled Clorox brand bleach and then returned to the free stall barn.

Milk samples were collected using an automatic milk sampling device. Milk samples were collected at three consecutive milking (08:30, 13:30, and 20:30 h) on Mondays during the first 10 wk of lactation. A set of 3 milk samples, one for each daily milking the each cow was saved in capped vials (10 mL) containing bromophenol blue and 10% preservative (DMF control system, Inc.) and analyzed at Serotec Dairy Lab (McDonough, GA) for contents of fat, protein, milk urea nitrogen (MUN), and the somatic cell count (SCC).

Statistical Analysis

Data collected during the experiment were analyzed in two sections. The first included data collected during the prepartum period and the second the data from the

postpartum period. Data were analysed using Proc GLM procedure as a nested design by least squares analysis of variance procedures of SAS (1991). Additionally, Mixed model was used in computer specific least squares means (Littel et al., 2006). Statistical analysis was performed for BWP and BCS, milk and 3.1 % BCM yields, and concentrations of BT, INS, IGF-1, glucose and NEFA in plasma. Time periods considered for data analysis were the prepartum period (-4 to -1 wk), overall postpartum period(1 to 4 wk) and the period from 5-10 d postpartum for MY. Models included the main effect of treatment (TET), calving month (CMO), their interactions (TET*CMO), and week-of days to the highest order significant for overall prepartum and postpartum periods, as appropriate.

Regression analysis was performed to the highest order significant up to quadratic order to check for the trends in measures during prepartum period for MY during the overall prepartum period. Tests of homogeneity of regression were performed to determine whether there was evidence that regression curves were not parallel (Wilcox et al., 1996). In addition, gross coefficients were estimated.

Specific models are described in the Results section. Significance was declared at $P < 0.05$, except where noted.

Results

One hundred ninety three cows were enrolled in the experiment, during year 1, plasma samples were collected from 44 Holstein cows (Control/C7-44 vs. hypercalcaemic/H7-40) and plasma samples were analysed for BT, IGF-1, INS, NEFA and glucose. During year 2, effects of MST on BCS and BWP of 114 Holstein cows (C-TT vs. 1-104,

were evaluated. During both years, milk yield data were collected and combined and analysed for the overall MY experiment.

The mathematical model selected for analysis of dependent variables during the prepartum [-3 and -1 wk for blood measurements, 0 and 3 wk for BCS and BW measurements] and postpartum (1, 4 and 8 wk for blood measurements, 2, 4, 6, 8, 10 wk for BCS and BW measurements and 0 to 100 d for MY), periods included main effects of season (TRT), calving month (CMD), the two-factor interaction of TRT*CMD and new(TRT*CMD). Weeks to the highest order significant up to cubic order for weekly measurements also were included.

The main effect of calving month resulted in two different groups:

- CMD 1= those calving in October 1998
- CMD 2= those calving in November 1998
- CMD 3= those calving in December 1998
- CMD 4= those calving in January 1999
- CMD 5= those calving in September 1999
- CMD 6= those calving in October 1999
- CMD 7= those calving in November 1999
- CMD 8= those calving in December 1999
- CMD 9= those calving in January 2000 and
- CMD 10= those calving in February 2000

During the experiment, 13 cows (year 1=6 cows, year 2=7 cows) from TRT 1 and 13 cows (year 1=6 cows, year 2=7 cows) from TRT 2 were culled at the end of the lactation because of breeding problems, chronic mastitis, or insufficient milk production.

Changes in Body Weight and Body Condition Scores

A major objective of this experiment was to evaluate changes in BW and BCS during the prepartum period starting about 3-4 wk before parturition and continuing

through 18 wk postpartum to monitor when the decline in BW and BCS shall be increasing values. During the period from wk 3 to day of calving no differences were detected between the treatment groups for mean BW and BCS (Figures 2-4 and 2-5). Least-squares means (LSM) for BW and BCS up to calving are in Table 2-2. No effects due to TET, CMD or two-factor interaction TET*CMD were detected for either BW or BCS, whereas significant linear effects of week were detected ($P<0.01$), Table 2-3). The mean BW -3 wk prepartum of cows in TET 1 was numerically less than for cows in TET 2 (796 vs 797 kg), but the difference was not significant. At the same time, the mean BCS of cows were 3.71 (TET 1) and 3.77 (TET 2) and did not differ due to treatment (Figures 2-4 and 2-5). At the week after calving (wk 0), cows in both groups had lost BW (652 vs 658 kg) and BCS (3.58 vs 3.59) (Table 2-2).

Least squares analysis of variance for BW and BCS during the overall postpartum period (1-35 wk) is in Table 2-4. The effect of TET on BW during this period was significant ($P=0.0144$), cows in TET 2 better maintained their BW during 35-wk postpartum (Table 2-2). No effects were detected for CMD or the two-factor interaction TET*CMD (Table 2-4). A significant linear effect of wk was detected for BW ($P<0.01$). Even though cows in both treatments had similar BW the week after calving (wk 0, 652 vs 658 kg), the loss in BW for cows in TET 1 was greater during the postpartum period (Figure 2-4). Two weeks following parturition cows in TET 1 had lost 8.2% of their BW compared to wk 0, whereas the loss of BW for TET 2 was only 3.6%. During the following 3 wk period, BW loss was less by cows in both TET groups. There was a slight but not significant increase in BW observed beginning wk 4

Table 1-1: Local System Means and SE of MBT and BCS all Days in Control and MBT Reported Groups During the Pre-report and Post-report Periods.¹

Day (Day)	Overall population		Day -28 to -J1		Overall population		Day 1 to J		
	LSM ²	SE	LSM	SE	LSM	SE	LSM	SE	
MBT	MBT 1 (p=07)	489	± 13.41	706	± 12.83	617	± 9.84	632	± 10.44
	MBT 2 (p=04)	707	± 10.85	737	± 11.86	640	± 8.79	688	± 10.88
BCS ³	BCS 1 (p=07)	340	± 8.04	371	± 9.86	333	± 9.85	350	± 9.84
	BCS 2 (p=05)	358	± 8.64	377	± 9.86	356	± 9.86	358	± 9.83

¹ MBT 1=pre MBT, MBT 2=to-day MBT (J1-J3 through J60), BCS=Local System Means.

² BCS estimated on a 1-5 scale (Barnhouse et al., 1987).

Table 2b. Continued.

	Exp 1a		Day 2b		Day 4c		Day 5d		Day 7e	
	LM	SE	LM ²	SE	LM	SE	LM	SE	LM	SE
2.3. 4a)										
TBT 1	504	± 9.04	614	± 9.48	645	± 9.87	818	± 10.28	423	± 10.04
TBT 2	549	± 8.26	835	± 9.34	678	± 9.42	561	± 9.92	608	± 9.58
BCS										
TBT 1	3.35	± 0.20	3.28	± 0.20	3.22	± 0.16	3.23	± 0.26	3.22	± 0.26
TBT 2	3.48	± 0.20	3.27	± 0.25	3.31	± 0.19	3.21	± 0.26	3.24	± 0.28

^a TBT from 160T, TBT 1a-1b, TBT 1c-21 through 4a-c; ^b 2000-L-204 Square Means
^c BCS referred to a 1.5 scale (Chen et al., 1989)

Table 2-4: Local Significance Analysis of Variance for TRT and MCS of Ethanol in Green Building Programs (Diversity 1 to 2 m)

Source	TRT			MCS		
	df	MS	F	df	MS	F
TRT ¹	1	9492.53	8.86	1	623.08	0.28
CMO ²	2	2769.81	8.23	4	173.02	0.43
TRT*CMO	2	2769.86	1.95	4	133.65	0.287
Corr-(TRT*CMO)	161	113952.45	11155.83	164	271307	8.71
TRT ²	1	52164.26	79.39	1	21024	71.81
Error ³	158	732.82		160	648.78	

¹TRT=Treatment (TRT or no TRT)

²CMO=Calming Month

³Residual

⁴Three times = Type I Error of Squares for TRT, both and Type III for others

Table 3-4: Least Squares Analysis of Variance for BQ² and BQ³ coefficients Data During Traffic Loading (0-1 hour)

Source	BQ ²				BQ ³			
	df	MS	F	PG>F	MS	F	PG>F	
Trial ¹	1	10734.12	3.28	0.0744	1.3099	1.32	0.2492	
Chord ²	5	13435.46	8.87	0.0117	1.8214	0.18	0.8328	
Trial*Chord	5	77682.58	1.74	0.2412	3.4979	0.47	0.2148	
Error (BQ ² +Chord)	10	1715403.42	36624.88	0.0006	60.3888	0.18	0.8008	
BQ ² ³	1	268.79	1.01	0.3096	0.7195	0.42	0.5208	
BQ ³ *BQ ²	1	15488.79	22.18	0.0004	0.2827	0.18	0.6813	
Total ⁴	410	281.43			0.2084			

Trial=Drumbeat (df=1) vs air (df=1).

² Chord=Curving Month

³ Trial.

⁴ Error term= Type I Sum of Squares for BQ² term and Type III for others.

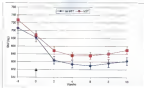


Figure 2-4 Least square means of body weight changes of Holstein cows during the preparturient and early postparturient periods (-4 wk through 10 wk). Arrow indicates calving.

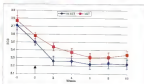


Figure 2-5 Least square means of body condition score changes of Holstein cows during the preparturient and early postparturient periods (-4 wk through 10 wk). Arrow indicates calving.

and at wk 8 the cows in TET II and TET I, respectively. Cows in TET II maintained significantly greater BW throughout the lactation period (Figure 3-4).

Trends for RCS of udder in the two treatment groups paralleled that described for BW (Figure 3-4). The RCS tended to differ during the lactation period ($P=0.14$). No differences were detected due to CMQ or the two-factor interaction TET \times CMQ (Table 3-4). Cows in both treatment groups lost RCS beginning at weaning (Figure 3-5). Cows in TET I had greater decline at wk 2 than cows in TET II. RCS loss from wk 2 to wk 3 was 4.8% and 3.3% for TET I and TET II, respectively. Beginning wk 6 both groups maintained their RCS; however, they had lost 4.0% and 7.1% of their RCS at that time, respectively compared to wk 0. However, RCS of cows in TET II at wk 10 increased slightly and RCS of cows in TET I was significantly greater at wk 10 (Figure 3-5).

Milk and Lactation Curve

Least square means for MY for period of time that milk was rejected (0-40 d) and the overall lactation (0-100 d) periods are in Table 3-3. Significant differences were detected due to treatment during the lact rejection period (0 to 40 d). Injured cows had significantly higher mean MY ($P<0.05$) during the first 40 d of milk than control cows ($P=0.002$). TET I=16.7 vs II=18.3 kg/d. No effects was detected due to the two-factor interaction TET \times CMQ. A significant quadratic polynomial effect of day was detected (Table 3-7). However, no difference was detected due to treatment for MY during the first 100 d of lactation (Table 3-7).

Table 3-3 Least-Squares Means and SE of Milk Yield, 3.5% FCM Yield, SCC, and Percentage of Protein, Fat and MUN in Milk of Holstein Cows During Early Lactation

Measurements	Treatments ¹					
	I			II		
Milk Yield (kg/d) ^{2a}	37.2	a	0.22	39.7	a	0.19
3.5% FCM (kg/d) ^{2a}	37.7	a	0.21	40.2	a	0.20
Milk Yield (kg/d) ^{2b}	33.9	a	0.09	36.2	a	0.07
Milk Yield (kg/d) ^{2c}	37.4	a	0.07	38.0 ^a	a	0.06
SCC ^{3d}	802	a	10.2	453	a	29.2
% Protein ²	3.08	a	0.01	3.01	a	0.01
% Fat ²	3.60	a	0.02	3.55	a	0.02
Total MUN ^{2e}	11.6	a	0.10	11.7	a	0.09

¹Treatment I=0 kg NITM; Treatment II=10.2 kg NITM.

^{2a}0-6 wk postpartum.

^{2b}0-10 d postpartum.

^{2c}0-100 d postpartum.

^{3d}SCC=Somers Cell Count x1000

^{2e}MUN=total Urea Nitrogen

^aP<0.1

^bP<0.05

Table 2-8 Least Squares Analysis of Variance for MMS and 3 1/4 PCMA Treats of Polystyrene Cores During Body Losses (1 Body)

Source	df	MMS Treat (log)			3 1/4 PCMA (log)		
		MSS	F	PC-F	MSS	F	PC-F
Treat ^a	1	1271.63	2.78	0.10364	1258.50	2.74	0.10316
CMO ^b	9	449.86	1.14	0.31119	464.31	1.46	0.1873
Treat*CMO	9	114.87	0.09	0.4909	628.82	1.68	0.17344
Core (Treat*CMO)	179	370.53	22.31	0.0001	179.26	14.82	0.0001
WGL ^c	1	6482.13	378.08	0.0001	2973.30	76.28	0.0001
WGL*WGL	1	3481.76	156.87	0.0001	3020.27	78.13	0.0001
WGL*WGL*WGL	1	144.56	3.62	0.0601	14.52	2.21	0.1403
Error ^d	1536	31.56			34.81		

^aTreat=Division (MST or no MST)

^bCMO=Clustering Method

^cWGL=Week

^dRow factor= Type 1 Sums of Squares for WGL term and Type II for others

Table 3-7. Least Squares Analysis of Variance for Daily-Mile Yield of Hauls Cans During Four Early Location Periods

Source	8-29 d			9-12 d		
	df	MS	F	df	MS	F
TWT ^a	1	10953.40	3.00	1	8334.10	1.20
CMO ^b	8	7548.95	2.11	8	8417.84	1.20
TWT*CMO	8	3484.31	0.97	8	4883.39	0.69
Cans (TWT*CMO)	121	3408.87	119.26	120	5030.85	103.46
Day	3	171779.33	2693.90	3	113710.96	1073.10
Day*Day	3	77343.33	2478.31	3	112090.55	1067.40
Day*Day*Day	1	16432.76	509.43	1	47533.35	1814.08
Day*Day*Day*Day	1	4342.71	139.81	1	18883.74	418.32
Day*Day*Day*Day*Day	1	3333.43	111.26	1	2558.46	71.10
Rep ^c	1	23.84		1	23.26	

^a TWT=Treatment (MT) or no MT.^b CMO=Cutting Month.^c Rep=Replicate Type I Series of Reps for Day term and Type II Series of Reps for other

Least squares analyses of variance for weekly milk and 3.5% FCM yields during the first 8 wk are in Table 2-4. Differences were detected due to treatment for both MT and 3.5% FCM yields ($P=0.004$ and $P=0.0005$, respectively). No effects were detected due to CMO or the two-factor interaction $\text{CMO} \times \text{TMT}$ for either measure of milk production. A significant cubic polynomial effect of wk was detected for each measure of milk production ($P=0.0001$ and $P=0.002$, respectively; Table 2-4).

Results indicated that cows injected with 16.5 mg hCG (TMT 2) had greater weekly mean milk and 3.5% FCM yields (39.7 and 46.2 kg/d, respectively) than unsprayed cows which were less (37.28 and 39.33 kg/d, respectively; Table 2-5). Figures 2-4 and 2-5. Increases in yields for MT injected cows (control) were 6-6 % for both measures. Least squares means for percent protein, percent fat and MT/fat for first 8 wk are in Table 2-5. During the first 8 wk no differences due to treatment were detected for percent protein, total fat, percent fat, and MT/fat. Means/SEs tended to be higher for cows in TMT 1 (400 to 453x1.0°).

Quadratic regression curves were calculated for the two measures of milk yield to describe the time trends for the individual treatments over 100-d lactation (Figure 2-6). Test of heterogeneity directed evidence that the curves were not parallel ($P=0.03$). TMT 2 had higher daily milk yield starting at the beginning of the lactation and increased greater throughout the 100-d measurement period than that of cows in TMT 1. However, diminishing responses of MT at 45 d resulted in a decrease in MT for cows in TMT 2 and MT for these cows was smaller in the time of cows in TMT 1 during the last 30 d (Figure 2-6) of the 100 d period.

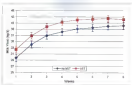


Figure 1-6 Least square means of weekly milk yields of Holstein cows during the first 8 wk of lactation

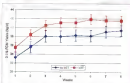


Figure 1-7 Least square means of weekly 3.5% FCM yields of Holstein cows during the first 8 wk of lactation

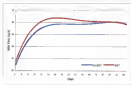


Figure 3.8 Quantia experimenters depicting MN of *Thalassia* coral during experiment.

Ramones, Growth Factor, and Metabolites

Preparation

Plasma concentrations of testosterone (BT) and PSA, growth factor (KGF-1) and metabolites (glucose and NEFA) also were evaluated during the period from -3 wk before calving through 3 wk postpartum (Figures 2-3 through 2-42). No differences in the mean concentrations of KGF-1, NEFA, or glucose were detected during the preparation period due to treatment. However, differences were detected for mean plasma concentrations of BT ($P=0.0004$) and PSA ($P=0.011$) during the same period (Tables 2-8 and 2-9).

The mean concentrations of BT on -31 d before expected calving did not differ for the two groups (6.1 vs. 5.4 ng/dl). One week before expected calving, which corresponded to the period following start of injections of MGT, mean concentrations of BT were greater for cows on TRT (3.0/3.7 ng/dl) whereas concentrations of BT for cows on TRT1 were similar to -31 d (5.8 ng/dl, Figure 2-8). Across treatments, significant effects of CMD ($P=0.0281$) and WC ($P=0.0082$) were detected but not due to TRT*CMD interaction during preparation period (Table 2-8).

Plasma concentrations of KGF-1 during the preparation period are in Table 2-9. Mean concentrations of KGF-1 did not differ during the preparation period. Significant effects of CMD ($P=0.0029$) and WC ($P=0.0282$) were detected but not for the TRT*CMD interaction (Table 2-9). Cows in control (TRT 1) and KGF injected (TRT 10) groups had similar concentrations of KGF-1 on 3 wk before parturition (3.26/3 and 17.1 ng/ml, respectively). Overall concentrations of KGF-1 decreased as parturition

Table 144. Liquid Biopsy Analysis of Mutations for Concentrations of 30, 100, and 300 ng/mL Plasma of Tumor Cells During the Progression Period (2.1 to 3.6%)

Experiments	30			100			300		
	df	LOS	P	LOS	P	PdP	LOS	P	PdP
TSC1 ^a	1	273.48	0.34	0.804	0.38	0.9784	85.00	0.06	0.1131
CD40 ^b	2	67.66	2.49	0.0281	0.13	0.2635	1871.46	3.11	0.0009
TERT-CD40	2	27.45	0.49	0.4922	0.16	0.2731	1431.45	1.12	0.3309
CD40(TERT+CD40)	18	27.72	0.49	0.4904	0.28	0.3861	1488.28	1.46	0.0298
VR ^c	1	254.39	12.46	0.0008	0.49	0.4944	1501.31	3.31	0.0340
Panel ^d	43	28.26		0.11			1501.27		

^aTSC1—Tumor Suppressor 1 (MTC)

^bCD40—Cell-Cell Molecule

^cVR—Value

^dPanel refers to Types I Series of Experiments for VR, term, and Type III for others.

Table 2.4: Least Square Analysis of Variance for Concentrations of PCBs and Chlorine in Plasma of Holstein Cows During the Pregnancy Period (25 wk to 1 yr)

Source	df	MSE			Observed		
		MS	F	P<=	MS	F	P<=
DAY ^a	1	6079.28	2.04	0.1679	47.72	0.40	0.527
CMQ ^b	2	34324.76	10.43	0.0001	170.33	1.34	0.2482
DAY*CMQ	2	110347.58	4.63	0.0281	338.20	1.21	0.3126
Sex (M/F*CMQ)	14	28790.88	1.21	0.1340	110.58	1.81	0.0687
SEC ^c	1	5089.07	0.53	0.4712	203.81	0.46	0.6368
Error ^d	143	21845.34			99.53		

^aDAY = Treatment (M/F or M/M)

^bCMQ = Cubic along Month

^cSEC = Secs.

^d Error term: Type II Error of Anova for SEC term and Type III for others

approached (wk -1) in both groups (254.4 and 166.7 mg/dL), this corresponded to 3.1 and 3.9% decreases, respectively (Figure 3-10).

During the preparation period, mean concentrations of INS in plasma was significantly higher in hST injected cows (TST-E; $P=0.016$, Table 3-8). Significant effect of WK ($P=0.044$) was detected, but not due to CMD or the two-factor interaction TST*CMD (Table 3-8). Mean plasma concentrations of INS tended to increase slightly (15%) from -3 wk to -1 wk in control group, whereas the increase was significantly greater for cows in TST-E at wk -1 (26% Figure 3-11).

Mean concentrations of glucose are in Table 3-9. Mean concentrations of glucose in plasma did not differ due to TST (56.3 vs 56.4 mg/dL). No effects were detected due to CMD or the two-factor interaction TST*CMD. A significant effect of WK was detected ($P=0.004$, Table 3-9). On wk -3, concentrations of glucose were 56.4 and 56.8 mg/dL for TST-E and C, respectively. However, on wk -1, glucose concentrations had increased slightly to 58.8 mg/dL for cows in hST injected group, whereas they were unchanged for cows in TST-E (56.1 mg/dL Figure 3-12). Although the slight differences in concentrations between groups on wk -1 were not significant, increase for hST injected group from wk -3 to wk -1 was significant ($P=0.03$, Figure 3-12).

During the preparation period, mean concentrations of NEFA in plasma did not differ between treatments (Tables 3-9 and 3-10). Significant effects were detected due to CMD ($P=0.004$) and the two-factor interaction TST*CMD ($P=0.005$), but not due to WK (Table 3-9). Plasma concentrations of NEFA tended to increase slightly (3.5%)

Table 2.18: Least Squares Analyses of Variance for Concentrations (IT, MS) and KPI-4 in Phases of Molecular Clones During Early Latencies (I to III only)

Source	df	MS			MS			KPI-4		
		MS	F	P-Val	MS	F	P-Val	MS	F	P-Val
TRT ^a	1	1162.14	18.19	0.0001	0.04	0.44	0.5345	201.2879	3.48	0.1001
CMO ^b	2	10.21	0.29	0.8589	0.16	3.12	0.1040	31299.17	3.79	0.0401
TRT*CMO	2	14.73	0.42	0.6577	0.09	1.28	0.2976	11439.70	3.10	0.0520
Clone(TRT*CMO)	74	46.11	1.46	0.0007	0.07	1.49	0.0001	79217.68	1.39	0.0005
WV ^c	1	37.09	0.60	0.4377	0.09	4.13	0.0438	21008.88	9.17	0.0020
Error ^d	128	45.41			0.08			14751.92		

^a TRT = Treatment (MS or MS-MO)

^b CMO = Cloning Month

^c WV = Virus

^d Error term = Type I Sum of Squares for WV, treat, and Type III for clones.

Table 2.11 Least Square Analysis of Variance for Concentrations of MBPA and Chlorine in Ponds of Helicon Creek During Early December (1-8 wk)

Source	MBPA			Chlorine		
	df	MS	F	MS	F	P-value
TREAT	1	402913.48	7.52	8.77	6.32	0.0218
CHLOR	2	340508.27	3.23	71.41	3.22	0.032
TREAT*CHLOR	2	148163.19	4.30	48.88	1.28	0.2546
One-(TREAT*CHLOR)	24	17328.41	1.13	30.31	1.28	0.0007
RES ^a	1	3110248.05	41.07	38.70	1.43	0.0007
Total ^b	158	30054.39		57.47		

TREAT=Treatment.

CHLOR=Chlorine Month.

RES= Residual.

^a Error term= Type I Sum of Squares for RES, term and Type III for others.

Table 2. (b) Linear Regression Means and SE of Parameters, Overall P-values and Molecular Concentrations as Means of Molecular Clones During the Preparation and Preparation Periods

Blood Measure	Treatment ^a					
	Preparation (1-2 to -1 wk)			Preparation (3 to 8 wk)		
	I	II	III	I	II	III
ST (ng/mL)	0.42 ± 0.12	0.70 ± 0.15	0.75 ± 0.15	0.35 ± 0.08	0.60 ± 0.08	0.88 ± 0.08
DOB (ng/mL)	0.15 ± 0.04	0.15 ± 0.04	0.14 ± 0.04	0.14 ± 0.03	0.14 ± 0.03	0.09 ± 0.03
SGA-1 (ng/mL)	180.5 ± 1.03	180.8 ± 1.4	180.7 ± 1.4	180.7 ± 0.1	180.7 ± 0.1	179.7 ± 0.1
Glucose (mg/dL)	84.2 ± 0.7	84.4 ± 0.7	84.3 ± 0.7	84.3 ± 0.9	84.3 ± 0.9	84.3 ± 0.9
MSHA (μg/L)	231.4 ± 16.5	230.8 ± 16.5	232.8 ± 21.1	231.1 ± 209.7	231.1 ± 209.7	231.1 ± 209.7

^aTreatment I-III: 1800, Treatment Break (mg/kg) 1800 (d -2) through d 80.

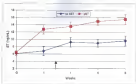


Figure 2-9: Least square means of concentrations of KT in plasma during the treatment period and through 8 weeks of lactation. Arrow indicates milking.

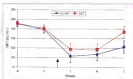


Figure 2-10: Least square means of concentrations of KT in plasma during the treatment period and through 7 weeks of lactation. Arrow indicates milking.

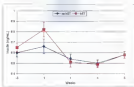


Figure 2-11: Least square means of concentrations of Insulin in plasma during the treatment period and through 8 weeks of lactation. Arrow indicates calving.

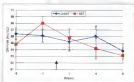


Figure 2-12: Least square means of concentrations of Glucose in plasma during the treatment period and through 8 weeks of lactation. Arrow indicates calving.

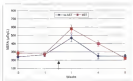


Figure 3-11 Last Square Means of Concentration of TILFA in Plasma During the infusion period and through 5 weeks of infusion. Arrow indicates infusion

from wk -3 to wk -1 for cows in TBT 1 (241 L to 315 \pm μ Eq/L), whereas concentrations tended to decrease for cows in TBT 2 (-3.3%, 255.4 to 279.4 μ Eq/L, Figure 2-13).

Interpretation

Another objective of the current study was to evaluate the metabolic response of cows treated with MET from parturition throughout the early postpartum transition period (2 wk) and through wk 8. A second series of analyses was performed to evaluate this time period. During the overall postpartum period, no differences were detected in mean concentrations of LMS or glucose due to treatment. Mean concentrations of IGF-1 tended to differ postpartum due to treatment ($P=0.083$). Significant differences were detected for mean plasma concentrations of SE ($P=0.006$) and NEFA ($P=0.003$) during the entire lactation period due to treatment (Tables 2-14 and 2-15).

Last square mean concentrations of SE differed significantly for TBT groups ($P=0.001$, Table 2-14). Cows treated with MET postpartum had greater concentrations of SE (14.6 μ g/mL, Table 2-12) than control cows (9.3 μ g/mL) and concentrations remained greater throughout the early postpartum period. After calving, mean concentrations of SE in control group (TBT 2) increased and plasma concentrations greater during this period (Figure 2-9). No effects due to CMD, VR, or two-factor interaction TBT \times CMD on SE concentrations were detected (Table 2-14).

For NEFA, no significant effects of CMD ($P=0.941$) or the two-factor interaction (TBT \times CMD, $P=0.963$) were detected (Table 2-15). A significant linear effect of VR on NEFA concentrations was observed ($P=0.023$). Overall, mean plasma concentrations of IGF-1 decreased following parturition for cows in both TBT 1 (-33%),

and II (11%) and remained low at wk 4. Differences between the treatments were not significant at wk 1 or wk 4. Mean plasma concentrations of LDL-C increased at wk 4 in TET-1 (21 %) and II (22 %) and differed between groups ($P=0.05$) (Figure 2.10).

During the postprandial period, mean concentrations of TG in plasma did not differ between treatments (Table 2.11). No significant effects of WE, CMD or the two-factor interaction (TET*CMD) were detected for concentrations of TG (Table 2.10). Plasma concentrations of TG decreased significantly after pretreatment in both groups and declined further at wk 4. However, at wk 1, concentrations of TG in plasma increased significantly for corn in both TET-1 ($P<0.01$) and II ($P<0.05$, Figure 2.11).

For glucose, no significant effects of TET, wk, CMD or the two-factor interaction (TET*CMD) were detected during the 1 wk postprandial period (Table 2.11). Glucose in both treatment groups showed stable or slightly decreased plasma concentrations of glucose after pretreatment and they remained lower in lactation steers (Figure 2.10).

Least squares mean concentrations of NEFA during the first 8 wk postpartum for TET groups differed significantly ($P<0.0001$). Significant effects were detected on NEFA concentrations due to WE ($P<0.0001$), CMD ($P<0.0049$) and the two-factor interaction TET*CMD ($P<0.0016$, Table 2.11). After calving, mean concentrations of NEFA increased for corn in both groups. Cows treated with TET tended to have higher mean concentrations of NEFA (375.8 μ Eq/L) than control cows (410.8 μ Eq/L.)

However, concentrations of NEFA had declined in both groups at wk 3 (TGT 1–249.2 vs TGT 1–122.2 $\mu\text{M/L}$, Figure 3–4.3).

Discussion

A series of adaptive adaptations normally occurs in cows during late pregnancy and the early lactation period. These adaptations are mainly characterized by increased hepatic gluconeogenesis to provide for increased use of glucose by the mammary gland during lactation, reduced glucose utilization in peripheral tissues, reduced peripheral utilization of amino acids, slightly increased mobilization of NEFA from adipose tissue, increased availability of glucose to support milk synthesis results from an overall decrease in its use by other tissues. Increased peripheral utilization of NEFA and lipids (triglyceride) is maintained with these changes (Bell, 1995). Reduced glucose utilization and low energy intake, due to the mother's large decrease in DM, maintain greater concentrations of NEFA and ketones (Peterson et al., 1994).

One of the major objectives of this experiment was to evaluate the changes in BW and BCS during 0–7-wk postpartum through 18-wk postpartum. Reports by Kott et al. (1991) and Gross (1998) indicated that cows not treated with MT had the greatest loss in BCS and they did not start recovery of BCS until 4-wk of lactation. However, cows treated with MT prepartum and postpartum had less pronounced losses in BW and BCS than cows not treated (Gross, 1998). In the current study, although prepartum BW and BCS did not differ between the treatment groups, cows in the MT-treated group maintained their BW and BCS better than cows in control groups following parturition. Recovery of BCS and gain in BW started at wk 4 for both groups

and cows in TRT II maintained higher BW from wk 2 postpartum through wk 10 postpartum (Figures 2-4 and 2-5).

Rapid and high rate of increase in DIM during early lactation is essential to provide energy and nutrients to support the rapid increase in milk yield. Cows treated with MGT (3 and 14 mg/d) during the prepartum-lactation, but not throughout the dry period, had greater DIM during the subsequent early lactation period (Joshi et al., 1991). Benneker et al. (1994) concluded that DIM tended to increase about 3 kg/d more in MGT-treated-cows (3 and 14 mg/d) after parturition. Joshi (1994) found that cows injected with ~5.1 mg MGT/d during both the prepartum and postpartum periods had greater DIM than untreated-controls, or than cows injected with ~5.1 mg kgBW only during the prepartum period, or only during the postpartum period. Results of Galey et al. (2000) also showed that greatest increases in DIM were by cows injected with 15.2 mg MGT/d during both the prepartum and the postpartum periods. Even though no direct measure of DIM was made during the current experiment, changes in both BW and BCS following parturition suggested that cows treated with 18.2 mg kgBW better maintained their DIM during the transition period. During current study, greater DIM of cows in MGT injected group (TRT II) likely was due to positive effects of MGT treatment on DIM, as typically occurs in lactating cows injected with MGT.

Another objective of this study was to evaluate the effects of MGT on decreasing if there was an effect on milk production due to MGT. The galactagogue response to exogenous reproductive MGT during lactation confirms that ST has an important role in dairy adaptations that occur during the transition from the non-lactating to the

lactating state. In the current study, a significant effect on milk production was detected due to treatment during the 60-d MST injection period. Increases in milk and 3.5% FCM yields were about 4.4% throughout the injection period. This also agreed with the findings of Sasmawati *et al.* (1991). They reported that slightly greater than a 4% increase in 3.5% FCM yields occurred when cows were injected with 3 or 14 mg/MST/d from 14-d prepartum through 60 d postpartum. Garcia (1988) reported that after 2 wk of lactation cows that had been injected with low doses of MST (<1 mg/MST/d) during the prepartum and postpartum periods (-21 through +60) had a greater and sustained increase in production during early lactation compared to cows in the other treatments (no-MST, or injected either prepartum or postpartum with 3.1 mg/MST/d). In addition to that, although not significant, because of small numbers of cows in each of the low treatment groups combined, Colby *et al.* (1980) found increased mean MY and yields in milk production when cows were injected with 16.3 or 15.2 mg MST/d beginning prepartum and continuing through +60 d postpartum. In another study, cows treated with MST (200 mg over 14 d period) starting 21 d prior to expected calving date through parturition produced 3.3 kg/d more milk than untreated controls during the first 42 d of lactation (Petersen *et al.*, 1999). Mosheim *et al.* (2000) concluded that MST injected early in lactation increased MY and DMI of cows after treatment with MST was started. On the other hand, increase in DMI was not enough to support the increase in MY and injected cows spent an extensive period of NEB which resulted in BW and BCS loss. However, in that study cows received a full dose of MST (200 mg/14d) which resulted in a severe NEB response in the injected cows. When cows were injected with

3 or 14 mg MST/d from 14 d postpartum through 60 DDM, they produced more FCM than controls (Kammarowala et al., 1994). In addition, cows receiving the lowest dose had higher pregnancy rate and higher conception rate than all other experimental cows and they also maintained BCS as well as controls. Eppard et al. (1996) failed to maintain MY when they injected Holstein and Jersey cows during the periparturient period with a full standard dose of MST. However, because cows also were used for milk fever induction and plasma concentrations of ST were low for treated cows in this study, this may explain results obtained.

Chelapp et al. (1993) reported a 4.7 kg/d increase in MY over control cows and a 1.03 point increase in the percentage when cows were treated with 50 IU MST starting at 4 wk of lactation. In the same study, feed intake of treated cows also tended to increase ($P=0.11$). In the current study, the treatment group that had the greatest BW and BCS also had the greatest milk yield. Even though cows in TRJ had the highest milk production, dose BW also was significantly greater ($P<0.05$, Table 3-4). Therefore, it seems very likely that milk production was supported to greater extent by increased DM due by more extensive tissue mobilization to provide the energy to support lactation because they also had less body condition during the same period.

Reduced DM immediately before and after parturition can limit energy and result in milk yield during early lactation. This limitation in milk production likely would be least for cows with greater DM. The group with the greatest DM during the treatment period and during the lactation would have greater quantities of energy and other nutrients needed to support maintenance of body tissues and milk production. As a

results, change in BW and BCS for the cows treated with MST would be less and less lipid and protein mobilization likely would occur. The MST treated group had greater BCS and greater production, which supports the conclusion that they had maintained higher level of DMI during the transition period, especially during the last week of pregnancy and first week of lactation.

Injecting low doses of MST had positive effects on concentrations of various hormones and metabolites when injected during ~ 31 d prepartum through ~40 d postpartum periods (Klaum et al., 2000; Calvey et al., 2008). Plasma concentrations of hormones (ST and IGF), growth factor (IGF-1) and NEFA also were altered during the time period that includes the defined transition period from 3 wk before calving up to 3 wk postpartum in the current experiment. Bouman and Verman (1993) reported that plasma concentrations of ST increased during late pregnancy with greatest concentrations at calving and during the early postpartum period. In the current experiment, cows treated with MST had higher concentrations of ST than control cows and they remained high throughout the early postpartum period (Figure 2-4). These results agreed with previous findings (Bouman and Verman, 1993). Lucy et al. (1999) reported that cows injected with MST during the postpartum period had increased concentrations of ST in plasma. Furthermore, cows injected prepartum with 25 mg MSTd had greater plasma concentrations of ST than untreated cows (Rushman et al., 1992). Prepartum injections of 5 and 15 mg MSTd increased plasma concentrations of ST which remained elevated (8.9 to 21.7 ng/ml) during the prepartum period, whereas

unpaired rows in the same study had low concentrations (< 4 ng/ml) during the same time period (Jansson et al., 1994).

Gonadotropin has a major role in regulation of IGF-I secretion and concentrations in plasma (Jansson, 1990). Gonadotropin release and circulating concentrations of IGF-I are primarily controlled with secretion of GnRH. Secretion of GnRH resulted in increased concentrations of IGF-I during both early and late luteal phase (Luoq et al., 1993; Shapiro and Reed, 1983). Compared with 19.2 or 33.7 ng/ml GnRH had greater concentrations of IGF-I than control rows that were not injected after parturition (Gulley et al., 2008). In addition, the IGF-I response to GnRH, as measured by concentrations in plasma, was greater when cows were in positive energy balance (Bachman et al., 1993). The current study failed to detect an increase in concentrations of IGF-I during the prepartum period. However, rows in this study were sampled only once after GnRH treatment (week -1) and this might not be enough time to provide or even to detect an increase in plasma concentrations of IGF-I, even if it had occurred. Additionally, the sampling time was closer to time of parturition at which time a decline in IGF-I concentration is found normally is expected. In the current study, plasma concentrations of IGF-I decreased around parturition (Figure 2-4) and after calving in both injected and noninjected groups of cows and this agreed with results of Baur et al. (1988) and Jansson (1993).

Although concentrations of LH remained elevated during the last week of pregnancy, plasma concentrations of IGF-I decreased during the prepartum period (week -1 to week -1). These results are similar to that of Jansson et al. (1994). They reported

that plasma concentrations of IGF-I did not remain high even though concentrations of IGF in plasma were greater from d -21 to d -10 postpartum. Low circulating concentrations of IGF-I during this time was associated with low nutrient intakes during early lactation (Yousef et al., 1990). Reduction of DM in growing steers decreased the basal concentrations of IGF-I in blood and suppressed the positive response of IGF-I to exogenous IGF treatment because response was usurped from IGF due to reduced intake (Finer et al., 1987). Low concentrations of IGF-I in blood during early lactation are associated with low DM during dry period (Rung et al., 1988). Although a decrease in IGF-I concentration was observed for both treatment groups in the current study, mean concentration of IGF-I for cows at T&T II was significantly greater at wk 8 (Figure 3-10). Decline in plasma concentrations of IGF-I after parturition in both groups might be a response to decreased DM experienced by the cows around the time of parturition. Thus, the increase in circulating concentrations of IGF-I in I&T exposed cows after calving might indicate better nutritional status once nutritional status has an important role in the circulating concentrations of IGF-I (Brewer et al., 1986).

During the prepartum period, mean concentrations of IGF in plasma were significantly higher in I&T exposed cows (Table 3-4). However, Buchanan et al. (1992) reported that plasma concentrations of IGF decreased as cows approached calving. On the other hand, results of the current study agreed with those of Yousef et al. (1990). They reported increased concentrations of IGF during late lactation and the dry period when IGF also was injected. Insulin concentrations of cows exposed with I&T or I&D

ing hGH also were greater than in interpolated control areas during the preparation period (Galey et al., 2000). High concentrations of IIG in blood were associated with persistence of the signs that had greater IIG, a feature that also promoted higher concentrations of glucose in blood during the dry period. However, during the preparation period, mean concentrations of IIG in plasma declined in two groups of cows that were in negative EB (Fries et al., 1994). The results of the current study agreed with findings that concentrations of IIG declined around parturition (Gries et al., 1998; Galey, 1998; Malven et al., 1987a). Decrease in IIG secretion and decrease in concentrations of IIG following parturition result in depression of lipogenesis (Moffatt, 1987). Despite the reduced concentrations of IIG, IIG receptor numbers increased in mammary tissue at parturition. In addition, during late pregnancy, increased mammary IIG causes a decrease in response to IIG on adipose tissue such that lipolysis and mobilization of NEFA were increased (Peterson et al., 1984).

Overall, access of XT to IIG should result in greater availability of glucose during the dry period to support milk synthesis during the upcoming lactation. Although the current study failed to detect a significant difference in preparation concentrations of glucose between treatments, the response was not $P < 0.05$ for cows in TET II (Figure 3-12) and significant within the TET group. Bromadiolone may have had a positive effect on plasma glucose concentrations during the time period evaluated which coincided with the time period when increased plasma concentrations of IIG were seen prepartum. This may have been affected directly via hepatic cells by promoting increased gluconeogenesis or indirectly by antagonizing effects of XT on

INS. Lactation is characterized by low concentrations of INS and a high ST INS rate. Insulinogen has a negative effect on ability of INS to inhibit gluconeogenesis, and it also upholds both INS uptake by the cell and the INS-protein necessary for the action of INS (Serchen et al., 1994). Insulinogen also has been shown to decrease the ability of INS to suppress gluconeogenesis (Serchen et al., 1994). These changes would narrow glucose production via gluconeogenesis and priority use of glucose for mammary tissues that could occur.

In the current experiment, no significant effects of TRT on concentrations of glucose in plasma were detected during postpartum period and decreased plasma concentrations of glucose after parturition were observed for cows in both groups. However, RT of cows in EST II averaged 2 kg/d more than for cows in EST I. This suggests there was a higher rate of gluconeogenesis and/or higher DMi by the EST treated cows during early postpartum period. These changes would support increased lact by providing energy and precursors needed for milk synthesis.

Mean concentrations of NEFA for TRT groups did not differ significantly during the postpartum period and no significant increase in concentrations of NEFA in plasma was observed for either treated or untreated cows. However, during postpartum period a different pattern was observed. Although cows in both groups had greater mean concentrations of NEFA, cows treated with EST had greater mean concentrations in plasma after calving. However, concentrations declined in both groups after calving and were slightly lower at wk 4 postpartum (Figure 3-13). As mentioned previously, lactation is characterized by low concentrations of INS and a high ST INS

rate. Decrease in DGE receptors and decrease in concentrations of DGE following parturition result in dependence of lipogenesis (deFeyere, 1987). Despite the reduced concentrations of DGE, DGE receptor develops greater sensitivity toward parturition. In addition, during late pregnancy there is increased resistance to DGE and this causes a decreased effect of DGE on adipose tissue resulting in increased lipolysis and NEFA mobilization (Petersen et al., 1994). Hall (1995) concluded that a combination of metabolic changes such as a decrease in de novo synthesis of TG, increased lipolysis, reutilization of fatty acids in the adipose tissue, and reduced intracellular reutilization of fatty acids among these lipolysis may cause increased mobilization of NEFA. High concentrations of NEFA in plasma also are associated with actions of some metabolic hormones. For example, high concentrations of ET in plasma during late pregnancy may reduce DGE receptors on adipocytes, inhibit the release of a novel messenger, or inhibit the DGE protein required for action of DGE. These changes will decrease rates of lipogenesis. Thus, ET can be considered a major regulator of metabolic adaptations during the transition period (McNamara, 1993). As indicated, ET is a primary homeostatic regulator during pregnancy and lactation (Rasmussen and Vernon, 1982), it regulates partitioning of substrate (carbohydrates, lipids, proteins, and minerals), and plays an important role in the coordination of various organs and tissues (Rasmussen, 1982). Nutritional status plays a major role in the regulation of lipid metabolism. When stores are in positive EE, synthesis and deposition of lipids in adipose tissue is related by ET which, in turn, stimulates nutrient utilization for milk production (Rasmussen and Vernon, 1993). Thus, ET reduces DGE

action, suppresses lipolytic enzyme activity, and reduces glucose uptake [Berman and Yarnon, 1982]. All these changes in actions of DMI during the transition period would support production of glucose from available precursors and conservation of glucose for mammary use by shifting peripheral tissues to utilization of other substrates available due to actions of ET and DMG. When cows are in negative EB, ET stimulation lipolyzes primarily by altering the sensitivity of adipose tissue to β -adrenergic input [Berman and Yarnon, 1982]. Therefore, for cows that are in negative EB and treated with MCT, increased lipid mobilization would be a major source of energy needed to support milk production [Joshi et al., 1982].

In the current study, cows treated with 10.2 mg MCT/d maintained their BCS and BW better. This suggests that these cows were less dependent upon their body reserves to support lactation than were untreated cows or those given a low dose of MCT. Maintenance of BCS and BW would be one consequence of greater DMG. Therefore, less of the energy and precursors needed to support mammary function during lactation would arise from their body reserves. To support milk synthesis, metabolism of other tissues is stimulated to provide the necessary precursors and energy sources. In addition to increased glucose production in the liver, glucose usage by other non-lactating tissues decreases. Although glucose is used as the primary energy metabolite and as a precursor for synthesizing milk constituents in the mammary glands, energy needed by these other body tissues can be derived from products of lipolysis. Therefore, lipolysis also must be an important pathway used by cows to

provide needed precursors in the early postpartum period, especially to supply the energy for milk production.

As indicated, ST acting as a homeostatic controller exerts important control over partitioning of nutrients and metabolism of substrates by various organs and tissues. Importantly, it appears to act on hepatic cells as well as on adipose tissue. Mobilization of proteins, minerals, lipids, carbohydrates and other nutrients is coordinated by ST (Bainman, 1992). Somatotropin exerts many differential effects on protein metabolism in both immature and non-immature animals.

Conclusions

Results of this study suggest that use of lower doses of hGH during transition period caused no additional negative energy balance as expected over compared to supported cows. Agonism of MT resulted in faster recovery of DCS during early lactation. Treated-cows produced more milk and 2.3% PCM during the lactation period. They also had higher concentrations of ST and IGF preparation and higher GH, IGF I and IGF II preparation. Thus, the changes in concentrations of metabolite hormones likely had a role in the positive effects on BW, DCS and MT that were seen for cows in T&T 2. However, no compound effects of hGH were detected on BW and the increase in BW during period after hGH injections were discontinued around 42 d postpartum. This could be due to the fact that preparation equivalent of hGH had no effect on a maternal effect on cell proliferation or amount of physiological tissue in the mammary glands. It most likely resulted in effect on the synthetic activity of these cells.

No apparent side-effect problems or propionate or propionic acidosis problems were observed for the cows across the treatments during the treatment period. Hence, it appears that cows could be treated with low doses of TMT to suppress TMT, even if they were to be exposed with the full dose of TMT (500 mg kg⁻¹ d) later in the lactation (after 60 d). No negative treatment effect was observed at the end of lactation, as determined by culling rates (TMT 1=13 vs. TMT 0=12 cows). This suggested that no detrimental effects occurred during the declining phase of the lactation. As a result, it appears that 10.2 mg/kg TMT of aqueous could be used during the immediate postpartum period and probably during the propionate period to suppress efficiency of milk production and suppress overall milk yields during early lactation. However, the effect of TMT suppresses propionate must be tested alone to fully evaluate any role it has upon subsequent health and production measures.

CHAPTER 3 FEEDING MANAGEMENT OF HOLSTEIN COWS DURING SHORT (DA) OR NORMAL (NR) DRY PERIODS

Introduction

Dry Matter Intake and the Transition Period

Although the maximum capacity to produce milk depends upon the animal's genetic makeup, age, physiological stage and environment, energy intake also is a primary limitation of milk production by animals, especially during early lactation (Wise and Jaeger, 1996). A significant decrease in DMI occurs during late pregnancy and continues into early lactation. The DMI is influenced by numerous factors such as physical limitations of rumen capacity, fat mass of animal, and metabolic changes and signals occurring during the transition period (Jeppesen and Anderson, 2000).

It has been suggested that the decrease in DMI during late pregnancy is caused by the pressure on the rumen by the growing uterus and contents, and the increasing requirements of skeletal fat (Wade, 1964). However, it is unlikely that the decrease in DMI is caused exclusively by rumen volume. Decreased rumen volume actually can be balanced, at least, by increased rate of passage of particles out of the rumen (Kane and Cook, 1987). In addition, diets having greater amounts of concentrates caused a greater decline in DMI during late pregnancy compared to a diet containing a low proportion of concentrates (Kagawa et al., 1974). Parbharwalla, Jeppesen et al. (1999) did not observe a rapid increase in DMI after calving as might be expected in terms of physical capacity of

the extent were the major factor determining DMI of ruminants. The increase in DMI follows the increase in ME (Shaggs *et al.*, 1993). As a result, it can be concluded that rather than physical constraints, metabolic and hormonal changes likely play a major role in regulating DMI.

Increased accumulation of lipids in body reserves during the prepartum period does regulate DMI (Innes *et al.*, 1984). Good body condition at calving is important for high producing dairy cows. On the other hand, overconditioning is not needed and should not occur during the dry period. Postcalving, cows with higher BCS lose more condition than cows with lower BCS, which also returns to positive energy balance faster (Clemmensen, 1986). However, a cow that is dried off with a BCS of 4.5 should be maintained at that level, because losing weight during the dry period increases subsequent incidence of metabolic diseases (Gibbell *et al.*, 1984). Rung *et al.* (1995) concluded that cows with higher BCS at calving lost weight for a longer time after calving than cows with moderate BCS, average condition lost was 0.80 from a 10 prepartum through 26 to 70 postpartum. Ingvorsen *et al.* (1997) concluded that there was a positive relationship between prepartum weight gain and the extent of postpartum mobilization of body reserves. They also argued that more than 40 kg BW gain during the dry period would deplete lipid intake postpartum and cause excessive mobilization of body tissue. However, association between adipose tissue and metabolic signals that determines appetite has yet to be fully described for cows.

Carpenter *et al.* (1998) associated increased glucose, protein and lipid drain results in mobilization of body fat and a rise in plasma concentrations of NEFA, glycerol and ketone bodies. In vivo, a negative correlation was observed between lipid intake and

circulating concentrations of NEFA, glycerol and ketone-bodies suggesting that these metabolites were potential signals for regulation of feed intake (Carpenter and Grossman, 1983). In dairy cows, 4-h infusion of lipids that provided 16.7 MJ of NE_{fat} resulted in a slight decrease in DMI postinfusion (Flume and Forrester, 1986).

It has been speculated that elevation of NEFA in the liver and the liver can decrease DMI. However, sustained elevation of NEFA in the retroperitoneal lipohyaloma for 14 d had no effect on feed intake or BW of rats (Baroncy and Wilson, 1991). When β -oxidation was inhibited by monoglycerolates, which depress long chain acyl-CoA dehydrogenase activity (Jung and Rorer, 1983), or by malonylglutarolates, which depress the carnitine palmitoyl acyl transferase-I concentration in the mitochondria (Hart et al., 1985), feed intake was maintained in rats having high fatty acid oxidation rates. On the other hand, Chou et al. (1987) observed a substantial decrease in DMI during the first 4 h postinfusion when they blocked fatty acid oxidation in dairy heifers by using sodium monoglycerolates.

It has been postulated that glycerol suppresses feeding and that it influences intake through a central nervous system mechanism. Intracerebroventricular infusion of glycerol to rats decreased their feed intake (Davis et al., 1987). However, only nonphysiological levels of subcutaneous glycerol injection influenced intake in rats (Carpenter and Grossman, 1983). Moreover, portal vein infusion of glycerol had no effect feed intake in ruminant milk sheep (Fisher, 1988).

As indicated previously, it has been suggested that blood metabolites and hormones alter feed intake. In rats, overeating evolved as a temporary increase in feed intake for 3 to 4 wk and that resulted in an increase in BW (Tortorella and Corda, 1973).

Injection of physiological doses of nitrogen increased these effects and a reduction of BW was observed as long as nitrogen (E_N) treatment continued (Tatelman and Gossard, 1973). Moreover, in vitro, submaximal injections of E_N decreased both DM and ME (Grossner et al., 1990). Progesterone (P_4), on the other hand, reversed the effects of nitrogen and stimulated feed intake (Wade, 1973). It has been shown that plasma concentrations of P_4 decline rapidly the week of calving and it is almost undetectable the day of parturition. Concentrations of nitrogen, on the other hand, rise by mid-gestation and peak prior to parturition (Claw et al., 1977). Intermittent injection of physiological amounts of 17β -oestradiol, in non-lactating cows and late pregnancy, caused a dose-dependent decrease in feed intake to various milk drops and in goats (Ingemansson and Andersson, 2000). It was suggested that E_N had direct effects on the paraventricular nucleus of the hypothalamus (Baker and Belkovich, 1989). Progesterone has not been reported to have a direct effect on feed intake (Ingemansson and Andersson, 2000). However, P_4 blocks the effects of nitrogen on feed intake in cows (Blair et al., 1972).

Corticotropin releasing factor (CRF) decreased DM intake central nervous system in cattle (Kuckenkorth and Molkart, 1986). Krahn et al. (1986) showed a partial reversal of CRF induced anorexia after central administration of a CRF antagonist. Because hypophysectomy had no effect on feeding or on CRF actions on feeding, ACTH and cortisol could not be the mediator of the decreased appetite (Larive et al., 1983). Furthermore, magnesium treatment did not influence intake in cattle (Hoad et al., 1979). Because concentrations of CRF are high several parturition, it may play a role in decreased intake of food around calving (Tucker, 1981). However, it is unlikely that CRF

has an important role in feed intake regulation during the early postpartum period because concentrations of CRF decline after calving.

Somatostatin (SS) plays a role in regulating feed intake (Ingraham and Anderson, 2000). Administration of the SS analog in the brain indicates that it is a central depressant effect of SS on feed intake in sheep (Bjorntorp and Wadell, 1989). Furthermore, administration against SS in growing cattle enabled them to consume more DM with a greater daily gain and improved feed conversion ratio (Ingraham and Anderson, 2000). Insulin also appears to play a role in long-term feed intake and weight regulation in ruminants (McCann et al., 1992). Acute peripheral infusion of INS that caused hypoglycemia also resulted in decreased feed intake in ruminants (Crombag, 1986) and humans (Datta and Whangwe, 1981). However, infusion of glucose prevented INS induced hypoglycemia (Sharp, 1974). Long-term infusion of INS via hyperosmotic osmotic minipumps implanted long-term (4-8w) in humans generally depressed intake of food, whereas short-term (4 hr) infusion under euglycemic conditions did not affect intake (Wadell and Bjorntorp, 1989). It is unlikely that INS plays a central role in DM during early lactation because concentrations of INS generally decrease after calving and are maintained at very low concentrations during lactation.

Central Regulation of Feed Intake

Although metabolic signals and hormones play major roles in regulating DM, physical and chemical characteristics of dietary ingredients and their interactions also are important. Physical regulation of DM occurs when feed intake is limited by the rate required for chewing or by discomfort of the gastrointestinal tract. Dietary factors that restrict eating time could result in decreased remaining time, which would restrict the

filling effect of the diet. The macronutrient (RR) generally in the diet is less distasteful when lower DM of nutrients (Allen, 2000). Sensory receptors and mechanoreceptors are located in the muscle layer of the wall of the RR, and are concentrated in the reticulum and caudal rumen (Leak, 1986). Epithelial mechanoreceptors are excited by light mechanical and chemical stimuli, whereas sensory receptors are stimulated by distention of the RR which provides information to the gastric system of the ruminant abomasum (Leak, 1986). These receptors are very sensitive to distension and can signal brain sensory centers to the end of the meal (Peters, 1999).

Both volume and weight of digesta in the RR are important for triggering distension. This was demonstrated by an experiment with steers offered a low quality forage diet (Selheim *et al.*, 1999). In their experiment, DM was reduced 11.2 g for each kilogram of weight and 157 g for each liter of volume that was added to the RR as diet fill. There are multiple mechanisms that regulate DMR. Both the neural strategy requirement and the filling effect of the diet regulate distension of the RR. However, physical regulation probably becomes a primary factor when the neural strategy requirement and filling effect of the diet increase. Added diet fill in the RR reduced DMR only when cows were in negative or slightly positive energy balance. Addition of diet fill into the RR had no effect on DMR of cows when energy balance was greater than 3.0 Mcal of NE_L per day (Allen, 2000). When energy limited cattle, NDF concentration was positively correlated with DMR. However, when fill limited cattle, NDF² was negatively correlated with DMR (Morrow, 1994).

The filling capacity of forage was inversely related to DMR (Hale and Campbell, 1982). The NDF content of forage was inversely related to DMR than other chemical

enzymes (Van Soest, 1982a). White (1980) suggested that NDF content was the best chemical factor to estimate the filling effect of forage. However, NDF alone did not predict the filling effect because other factors such as particle length, particle density, and rate and extent of NDF digestion were important contributors (Morris, 1984). Initial density of forage was related, in part, to NDF content (Morris, 1985). However, the filling effect of a diet also was dependent upon factors affecting rate of digestion and passage from the rumen. Thus, the rate and extent of digesta passage, RER activity, functional characteristics of microbial consortia, and rate of emptying of the abomasum also determined the filling effect (Allen, 1984).

Low ruminal pH can decrease fiber digestion and increase filling effect of the diet. Rate of starch digestion also can have significant effects on DMI of cows. Increased ruminal starch degradation, as a percentage of DMI, resulted in significant depression of daily DMI of cows. This may have been due to increased acid production in the RER. Increased ammonia could be another reason for reduced DMI. Epithelial receptors in the stomach and/or cranial set of the rumen are stimulated by acidic stimuli and hyperosmotic solutions. Thus, direct stimulation of receptors by hyperosmotic solutions can stimulate satiety. In addition, increased ruminal starch degradation improved propionate production (Allen, 1986).

There is substantial evidence that propionate affects satiety. And and Ferrel (1988) reported that there were receptors in the liver that were sensitive to propionate. Depression of DMI by propionate was disrupted by spleen blockade, by hepatectomy, and by total liver denervation. Ferrel also fiber digestion in the RER and it also can affect cholesterol. High diet that stimulated cholesterolemia (CC/C) increases,

which represent DM) by reducing RA activity and the rate of passage, tended to also depress DM digestibility via decreased fermentation. The crude protein (CP) of diet, on the other hand, often was related positively to DM of excreta. As dietary CP increased from 15 to 17% and NEL from 1.58 to 1.54 Mcal/kg of DM, there was a 30% increase in DM in the excreta (Henry, 1983). Although CP had a positive effect on DM, this effect decreased exponentially as the percentage of CP in the diet increased (Allen, 2000). One unit increase in diet CP content resulted in nearly a 0.7% kg/d increase in DM at 12% CP in the diet (Boffler et al., 1984). In the same study, there was only a 0.04 kg/d increase in DM at 18% CP in diet (Boffler et al., 1984). The positive effect of CP may be due to a reduction in passage rate, as protein was substituted for starch in the diets and increased dietary crude units also can increase the rate of clearance of metabolic fluids from the blood, increasing hunger and reducing the retention interval. Another mechanism that could be involved is that increased RCP affects digestibility of foods (Owen, 1944). Presumably there are reductions in retention in fiber and DM digestibility excreta (Allen, 2000). However, there were no differences in DM found between feeding RCP and BCP.

NET and Tissue Protein

Treatment of swine with NST during lactation was approved for use in US during February 1954 by the Food and Drug Administration (FDA). Since then it has been used extensively. In addition, regulatory agencies in 34 countries have marked similar conclusions in the US agency with respect to food safety and 24 countries approved use of NST, namely Algeria, Brazil, Bulgaria, Colombia, Costa Rica, Czech Republic, Ecuador, Hungary, Jamaica, Kenya, Korea, Malaysia, Mexico, Nicaragua, Pakistan, Peru,

Romania, Russia, Slovakia, South Africa, Turkey, United Arab Emirates, Ukraine and Zimbabwe.

After over a year of use-of-MST in the US, MST had been given to 235,000 acres in New York (40% of the state total), the major dairy state in the eastern US. USDA data showed that during the first 10 mo of 1994, fluid milk consumption increased by 1% compared to 1993 (pre-MST), milk prices received by farmers did not plummet but increased slightly. Farmers using MST generally increased their productivity and for them large farmers using the technique exclusively and driving small farmers out-of-business as even was predicted it would, the size of farms that adopted use of MST closely resembled the distribution of land area owned in the US. About 50% of all sales of MST have been to farmers with 100-or fewer acres (Hartwell, 1994). In January 1994, of nearly 6 million dairy cows in the US, about 20% were on MST treated lands, and 100 additional dairy farmers a month were reported to be adopting use of MST. The average dairy farmer used the commercial MST product (TOSILAC[®]) to supplement more than 50% of the feed at any one time, depending upon individual herd management practices and stage of the adoption.

The upsurge in use during the lactation cycle of cows is that use-of-MST is at about peak MT (~ 0.03 d) and is not continued throughout the remainder of the lactation (Chakrapa and Gillingham, 1993). Milk yield gradually increased over the first few days following MST treatment and reached new mean during the first week. Despite large increases in milk production, feed intake did not increase immediately following MST treatment. Early production responses therefore, were due mostly to partitioning of

movements away from body towards stationary placenta to support uterine milk synthesis.

Although increased MY responses typically are found when cows are exposed with MFT beyond 40 d postpartum, no such increase in MY was observed when exogenous MFT was injected during prepartum and early postpartum periods, which included the so-called transition period. Ross and Hest (1982) speculated that increase of delayed increases in OMS, treatment with MFT during the transition period might lead to more manual health problems such as lameness, fatty liver, wasting, and increased susceptibility to other diseases, or it could result in lower than expected MY responses. Indeed, by Lipard et al. (1994) failed to show an increase in MY when they injected Holstein and Jersey cows during the prepartum period with a daily standard dose of MFT (PGU/LAC/4). Thompson et al. (1991) administered GPR prepartum to beef heifers to increase secretion of BT before parturition and during early lactation. Treated heifers lost more BW and had delayed ovarian activity, whereas no difference was observed in MY. In another trial, Holstein cows received 0, 3 or 14 mg MFT/d during the last 40 d before parturition (Chenoweth et al., 1994). Cows treated with 14 mg MFT/d had increased yields of whole-mounted milk (SCM) early during wk 1. However, they found no differences in SCM among treatments. Except for the cows treated with 3 mg/d of MFT during wk 10 of lactation, RM was negative for all cows during the first 70 d of lactation. Bachmann et al. (1995) evaluated whether a dose of 15 mg MFT/d administered prepartum affected postpartum MY of Holstein cows. After covariance adjustment for previous milk lactation MY, 3.5% PCM yields of treated and control cows did not differ. They concluded that MFT treatments during prepartum period did not have either a positive or negative effect

on MY during the following lactation. None of the studies that used hMT treatments during the transition period have reported any animal health problems such as ketosis, dry liver, mastitis, and increased susceptibility to diseases in the treated cows.

Barbarger et al. (1994) administered 20 or 40 mg hMT daily to Holstein heifers during the last trimester of their pregnancy. They found a significant increase in BCM production of the heifers injected with 20 mg hMT/d but only after 90 d of lactation. Pridmore et al. (1999) reported a significant effect of propionate/hMT treatments on milk production during early lactation which appeared to increase as lactation progressed. The strongest hMT injections (300 mg over 14 d period) were initiated 21 d prior to expected calving date and were continued until parturition. Cows treated with hMT in their trial produced 3.3 kg/d more milk than untreated controls during the first 42 d of lactation. However, cows in the hMT treated group had significantly higher initial BCS than the controls when they were assigned to the trial. This allowed treated cows to maintain more body reserves than controls and to lose more BCS without a negative effect due to hMT. This is a very important factor because hMT use likely increases negative energy balance and greater loss of body weight which supports the greater milk production and the DMI increase.

As reported for propionate treatments, early postpartum experience of hMT also gave inconsistent results. de Boer et al. (1990) injected cows with 20 g mg hMT/d during 4-5 d postpartum. No significant differences manifested the MY of control vs. hMT treated cows. Unfortunately, the cows assigned to hMT had lower MY potential based on the rate and extent of decline in MY after cessation of hMT injection. Thus, hMT experience enhanced MY to levels similar to those of controls (de Boer et al., 1990).

Mason et al. (1996) studied the mechanisms by which Cu scope of dairy cows and milk affected production and reproduction of high producing cows. They reported 160 mg of MT every 14 d from 10 to 150 DIM. Milk yield during the first 60 d did not differ between treatments. However, MT treatment significantly reduced MY beyond 60 d and peak milk production increased for treated cows. On the other hand, the effect of treatment on DCS was mixed. The DCS of treated cows decreased more and was considerably less for treated cows and progenies conception rate was affected adversely compared to untreated cows. Santos et al. (1998) compared effects of MT on the performance of early lactating cows fed diets differing in rumenally degradable starch. Ruminants were provided intravenously injection of 300 mg MT for 10 d starting at 5 DIM. A positive MY response was observed during the first 45 d and for the total treatment period (90 d). Increasingly, MY response to MT was less from 7 to 13 wk than from 3 to 6 wk. Higher ES and DCS were observed in this study. Authors concluded that the response to MT was less than usually observed for cows when MT injections began at peak MY. In another study, the same dose of MT was injected every 14 d from 10 to 150 DIM (Mason et al., 2000). They concluded that MT injected early in lactation increased MY but at the expense of an extensive period of MLH and BW and DCS decrease despite an increase in DIM after treatment with MT was noticed.

Richard et al. (1983) reported a 1% increase in MY when cows were injected with 30 g of MT starting 20 d postpartum. Milk fat also was elevated by 25%. In the same trial, when cows were injected beginning 9 d, MY response was greater (17%) with no change in milk fat. Chalupa et al. (1983) treated cows with 30 g MT starting at 4 wk of lactation every seven fed a diet that contained 0 or 1.1% sodium bromate. They

reported that MET treatment increased MY by 4.7 kg over required cows and there also was a 1.03 point increase in milk fat percentage. Feed intake of required cows tended to increase (17.4 kg vs. 16.1, $P=0.11$) in view of the larger results, Stammaridis et al. (1999) reported 3 mg or 14 mg MET/d from 14 d prepartum through 10 DIM. Cows that received either 3 or 14 mg of MET/d produced more PCM than controls, but PCM of the two MET-treated groups did not differ. However, cows receiving the lower dose (3 mg/d) had higher pregnancy rate and higher conception rate than all other experimental cows. Cows receiving 3 mg MET/d also maintained BCS as well as untreated controls.

Variable results within and among trials that evaluated use of MET either pre- or postpartum period may have been due, in part, to differences among the doses, that the animals were fed, or due to differences in BW and BCS of animals. Usually, a high dose of MET increased the length of time cows were in NDR, loss of BW and BCS occurred even though an increase in DIM might have occurred. That adequate BCS (3.5–3.75, Scott et al., 1983) for cows expected with MET pre- and/or postpartum is required because the cows require good management and adequate nutrition to produce and reproduce well.

Treatment with MET during both prepartum and postpartum periods likely caused metabolic changes after parturition that were beneficial to health and performance of the cows (Finley et al., 2006). In their study, pre- and postpartum exposures of 15.3 mg MET/d increased DIM of cows after parturition and there was less decrease in BCS and BW. This allowed the cows to recover to satisfactory BW and BCS more rapidly during early lactation. Cows also produced numerically greater daily MY and 3.5% PCM. In addition, Gaines et al. (2008) reported that exposure of 3.1 mg of MET/d before and after

periods increased DMI, MEV and efficiency of milk production during the early lactation period (200d).

It also is likely that treatment with hCT during both prepartum and postpartum periods caused metabolic changes after parturition that were beneficial to cows during the lactating phase. Evidence suggests that changes in circulating concentrations of hormones, growth factor and glucose were beneficial. Cows treated with 10.2 or 15.2 mg hCT twice and after parturition showed increased concentrations of SE, IGF-1 and T₃ in plasma (Goffe et al., 2000). Thus, the changes in concentrations of metabolites hormones likely had a role in the positive effects on DMI, MCS, BW and MEV of these cows.

Ammonia Diet

Milk fever is a common metabolic disorder in dairy cattle that generally affects older, higher producing cows. The majority of milk fever cases occur within 48 to 72 hr after calving, although cases may occur later in lactation. It was estimated that 3 to 10% of cases are affected by this disease with some herds having a prevalence as high as 50% (Eppert et al., 1999). On the other hand, the incidence rate of milk fever among lactating Friesian-type cows is less than 1% (Birk and Grober, 1982). The onset of milk production decreases the cow's blood Ca levels and she is unable to rapidly or completely replace this Ca. The body has reduced capacity to mobilize sources of Ca in bone and therefore is dependent upon the ability to absorb Ca from the gastrointestinal tract. As a result, hypocalcaemia occurs and decreases the cow's muscle contractions and rumen motility (Goffe et al., 1993).

The key to prevention of milk fever is management of the close-up dry cow group. Recommendations for the prevention of milk fever traditionally have included the proper feeding of Ca and P, especially during the late lactation and dry periods. More recently, however, dietary acidity and alkalinity have been associated with controlling the incidence of milk fever. Increasing dietary K from 1.0% to 1.1% decreased the incidence of milk fever from 18.6 to 8.0% in Jersey cows. Potassium concentration in forage plays an important role in incidence of milk fever (Goff et al., 1997). Addition of anion content to prepartum diets causes a metabolic alkalosis and this suggests that bone resorption of Ca is inhibited in cows fed high K or Na diets because feeding these diets results in increased blood and urine pH (Goff et al., 1997; Hart et al., 1994). Mild metabolic alkalosis caused by feeding anion diets increases the absorption of Ca from the intestinal tract (Goff et al., 1997).

Research into the dietary cation-anion difference (DCAD) of pre-calving dairy calves has been extensive during recent years. A major achievement has been that modifying the diet can allow feeding of high Ca diets without causing hypocalcemia (Gandy et al., 1995). Calculation of the DCAD is based on the levels of cations (K⁺ and Na⁺) and anions (Cl⁻ and S²⁻) in the diet (DM) (Hart et al., 1994). The most commonly used formula to calculate DCAD is the sum of the positively-charged ions (K⁺ and Na⁺) minus the sum of the negatively-charged ions (Cl⁻ and S²⁻) in the diet, as shown in the following formula:

$$\frac{mEq(K^+ + Na^+) - (Cl^- + S^{2-})}{100 \text{ gms DM}}$$

Positive DCAD diets can have a systemic alkalinizing effect on the animal. High levels of Na and K in the ration cause the blood to be slightly alkaline and this reduces the effectiveness of parathyroid hormone (PTH). Diets with a negative DCAD, on the other hand, are acidogenic. Acidogenic diets fed to dry cows have significantly decreased the incidence of milk fever (Black, 1984; Hirst et al., 1984). For dry cows, lowering the DCAD level to -40 to -50 mEq/100 g DM results in an inflow of negatively charged ions (phosphate and sulfate), usually the cow attempts to maintain electrical neutrality at the expense of available sodium. Hydrogen ions are generated to neutralize these negative ions, resulting in a mild metabolic acidosis. With chronic subclinical metabolic acidosis, there is inhibition because it is a reserve for carbonate ions, which serve to buffer the pH. In the presence of increasing CO_2 , Ca (and P) are mobilized from the bone and also Ca absorption from the GIT is enhanced. Chronic subclinical metabolic acidosis also increases urinary excretion of Ca, and as a result Ca retention decreases, which causes formation of $1,25(\text{OH})_2\text{D}_3$ and release of PTH to further stimulate bone mobilization. The mechanisms used to increase blood Ca are therefore in an activated state at time of calving, and the end result is a higher level of Ca in blood (Gagione et al., 1989). Steele et al. (1991) recommended that cows should not be on the negative diet for more than 4 wk before calving and the DCAD should be returned to the normal positive postpartum level after calving.

With variations in large and anion/cation ratios, some pH monitoring has become an essential tool to determine if the laboratory analysis and calculated DCAD of the ration, in fact, is correct. Alkaline diets produce urine pH of 8.0-8.3, whereas acidic

nutrient concentrations to fall to a level even less. If the pH falls to 5.7 and below, there is the danger of oxidative rancidity (Goell, 1958).

In order to formulate a diet with 10 to 15 mEq/100 g DM, it is essential to analyze all feeds for macroelement content (Ca, P, Mg, Na, K, Cl), and (S). Feeds ingredients with a low DCA/D, especially legumes, should be selected. If the final DCA/D is greater than +20 mEq/100 g DM, large amounts of anionic salts must be incorporated into the diet and this can result in reduced feed intake problems because the ration is too palatable (Hume et al., 1984). Adding appropriate amounts of (magnesium sulfate, potassium chloride, ammonium sulfate, sodium chloride, sodium sulfate) is very important (Moore et al., 2003). Magnesium sulfate is recommended as the first addition because it appears to be the most palatable, and because it can be used to meet the cow's requirement for magnesium. Doses between approximately 1,500-3,000 mEq should be added from salts, because there is a risk of acute rickets when doses exceed 3,500 mEq (Block, 1984). Finally, chloride sources (ammonium chloride, or magnesium chloride) can be added to bring the DCA/D to 10 to +15 mEq/100 g DM. Formulations for phosphorus intake should be considered and done carefully and also should ensure the requirements for other nutrients are met (energy, protein, vitamins, and minerals) (Cloutier, 1983).

Advantages of Short-Duration Periods on Feed Intake

The surface of the rumen mucosa is characterized by ruminal papillae which can be defined as organs of absorption (Van Soest, 1982). Their distribution, size and number are closely related to feeding habits, forage availability and digestibility. The typical features of rumen papillae are genetically fixed but may vary considerably under different

feeding conditions. This can result in acute and usually temporary or seasonal adaptations (Van Soest, 1982). For example, increasing proportions of hays and greenens made maintain the natural fibre flow which stimulates interest in the rumen resulting in vascular budding and epithelial cell proliferation. Thus, there are differences in number and rate of papillae within the rumen. Lower energy diets fed during the early dry period may reduce the absorptive area of rumen as much as 50% during the first 7 wk of the dry period. Changes in the numbers of ruminal papillae occur in response to nutritional changes. Complete adaptation of ruminal papillae requires a period of 2 to 3 wk (Dobson *et al.*, 1985; Goff and Rens, 1987).

Microorganisms in the rumen depend upon the animal to provide the physiological conditions necessary for their existence. In fact, these microorganisms are essential for digestion and fermentation of large amounts of fibrous feeds which the ruminant consumes, but otherwise cannot utilize efficiently. Thus, by providing a variable and constant environment for these microorganisms, the ruminant is able to utilize the end products of fermentation to meet its own nutritional needs. Comparison of rumen microorganisms shows that there is a high level of variation. The large diversity in the types of microbes found in the rumen reflects, to some extent, the fact the rumen consumes (Van Soest, 1982). Growth of microorganisms and efficient fermentation of feed by microorganisms depends upon a constant and suitable environment. Changes in feed and feed composition, as well as rumen pH, not only reduce shelf life microorganisms in the rumen but also decrease the efficiency of the fermentation and absorption of end products of the fermentation.

Changing the diet of the animal population is period of transition in the human nutritional population. The proportions of the different species in the human diet shift to a new balance, one which has accommodated the dietary change and is referred to as adaptation of nutritional population. For example, exposure of a lean energy-dense diet upon drying-off causes a shift in nutritional population and the population of lactones that are capable of utilizing lactate to uric acid, propionate, or long-chain fatty acids declines. Adaptation of the lactone converting bacterial population is slow and may take several weeks to occur (Yokoyama and Johnson, 1983).

The current standard dry period of 60 d essentially requires dairy managers to feed dry animals in different phases; most recently termed as late-off dry period (FODs) with proportionally and close-up dry period (CUDs) with proportionally. During these periods, data fed to animals will vary because of metabolic differences of cows during these short time periods. The changes in data fed to cows progress from the lactation diet to FOD then FOD to CUD and CUD to early lactation diet allows the cows and its microbes to adapt these times during a relatively short time period when these changes in feed intake are occurring. It is likely that these changes may, in part, be a result for reduced DMI and will lead to further decreases in the DMI that occurs during the late CUD period especially during the time close to parturition. This may limit the increase in feed intake that should occur shortly after parturition. Early lactation is the time period when most rapid increase feed intake and more efficient fermentation /utilization of ingested feed are desired (Drouilly, 1999). If the length of dry period can be decreased to ~30 d, then it may be possible to develop a better feeding program to move cows to feed earlier. Formulated using the same constraints as in the lactation diet. This would require making

breast changes in the dams that are fed and could adversely affect pupal development and prevent complete adaptation of the microbial populations of the rumen near to calving (Cromar, 1991). This will encourage maintenance of the desired rumen population, better rumen development, and greater per-partum metabolism of C₄. Thus, it is possible that cows will increase feed intake faster, have better efficiency of fermentation and absorption of and products of fermentation, and better resistance to metabolic disorders during early lactation.

The major objectives of this research were to evaluate dry-period length, the type of prepartum transition diets (concentrate or forage) and supplemental ruminant NMT during the transition period to improve DMI, DCS, milk production and cow health during lactation.

Materials and Methods

Experimental Animals

Eighty seven multiparous Holstein cows were utilized in this experiment. Cows were selected randomly from the Dairy Research Unit (DRU) herd of the University of Florida approximately 8-9 wk before expected calving date. Cows were assigned to one of two treatment groups) 60 or 30-d dry period length. Cows with longer dry periods were dried off and moved to the dry herd of the DRU and fed the level FCG diet, whereas cows with shorter dry-period remained in the milking herd. Approximately -30-d before expected calving day, cows in the shorter dry period groups were dried off. Cows in both groups were housed and managed in a free-stall barn and treated to one electronic feed gate (Ammann-Cohen, Inc., Northwood, NH). They were assigned to the individual gates randomly, fed concentrate diet (Table 1-1) then treated to one gate for one week before

urine measurements were recorded. Three weeks before expected calving, cows were assigned to either an intense CLD diet or assigned to the control CLD diet (Table 3). Cows assigned to trial completed the experiment and were returned from trial over a 300 d period from September 2000 through June, 2001. The body weight (BW) and BCS of the cows at the time they were assigned ranged from 575 to 670 kg and 3.08 to 4.11, respectively. When cows were assigned to treatments, no differences were detected among treatment groups for mean BW (± 40 kg) and BCS (± 1.15).

Experimental Design

Cows were assigned randomly to a 3 x 2 x 2 factorial arrangement of treatments. Treatment group 1 (21 cows) was a 10 d-dry control group that received no external oxytocin treatment (ECP, Pharmacia & Upjohn, Kalamazoo, MI), cows in group 2 (21 cows), and 24 (24 cows) had 10 d-dry period and received 10 injections of ECP or saline (saline) (see ECP) respectively at the time they were dried-off. Each of these three treatment groups were further divided into two groups, one-half of each were injected with MST (0.4 mL MST; POSELAC®) (weekly) and the other half not injected. The quantity of POSELAC® provided approximately 19.2 mg MST/L, and injections were continued up to 60 d postpartum. After 60 d, all cows on experiment received the full dose of POSELAC® (384 mg) weekly. One-half of each of the three main groups were fed either alfalfa or lucerne diet during last 3 wk preparation. Urine pH of the cows was measured routinely (twice) during the preparation period. One cow from 60 d dry period MST injected and fed alfalfa diet preparation, one cow from 10 d dry no ECP, no-MST injected and fed alfalfa diet preparation, and one cow from 10 d dry ECP injected, no-MST and fed alfalfa diet preparation were removed from the experiment due to

Table 3-4. Dry Matter Concentration and Chemical Composition of *Arundo* and *Cortaderia* CUD Past in Pasture Cows¹.

	<i>Arundo</i> -CUD	<i>Cortaderia</i> -CUD
Cum Silage	41.48	40.96
ADFM Silage	—	5.90
Cum Hay	33.83	18.28
Concentrated Hay	18.64	11.17
Haylage Meal	8.88	8.18
Concentrated with E and	5.14	5.26
Protein	2.43	2.35
Springer Moisture	6.44	2.80
Sub	0.48	0.50
Subsist Concentrate	—	0.54
Chemical Composition	Percentage ²	
DM	43.84	42.97
CP	14.87	14.88
Std CP ³	28.11	28.28
ADF	33.93	28.17
NDF	37.25	40.10
EE ⁴	4.18	5.41
TDN	67.55	67.20
NE _L (Mcal/kg)	1.94	1.82
E	1.83	1.14
St ⁵	0.27	0.46
Cl	1.88	0.62
S ⁶	0.24	0.10
Ca ⁷	1.48	1.07
P ⁸	0.25	0.18
Calculated OERD ⁹	18.8	15.4

¹From MIEBWA Forage Laboratory, Hays, KS, analyses of components. ²DM base. ³Percentage of the CP. ⁴Extraneous. ⁵Metabolizable DM.

postabdominal, uterine incision and dissection of abomasum, respectively. A total of 64 cows completed the experiment. Actual days dry for 30 d dry no-BCP, 30 d dry BCP and 60-d dry cows were 35.6, 35.5 and 44.1 d, respectively.

The 3x2x2 arrangement of treatments resulted in twelve treatment groups. The experimental arrangement of treatments (T), dry periods, date and cow numbers in groups follows:

Treatment I	7 cows, 60 d dry period, 0-BCP, constant diet proportions.
Treatment II	7 cows, 60 d dry period, 0-BCP, constant diet proportions.
Treatment III	7 cows, 60 d dry period, +BCP, constant diet proportions.
Treatment IV	6 cows, 60 d dry period, +BCP, constant diet proportions.
Treatment V	7 cows, 30 d dry period, BCP, 0-BCP, constant diet proportions.
Treatment VI	7 cows, 30 d dry period, BCP, 0-BCP, constant diet proportions.
Treatment VII	6 cows, 30 d dry period, BCP, +BCP, constant diet proportions.
Treatment VIII	7 cows, 30 d dry period, BCP, +BCP, constant diet proportions.
Treatment IX	7 cows, 30 d dry period, Constant diet, 0-BCP, constant diet proportions.
Treatment X	7 cows, 30 d dry period, Constant diet, 0-BCP, constant diet proportions.
Treatment XI	7 cows, 30 d dry period, Constant diet, +BCP, constant diet proportions.
Treatment XII	7 cows, 30 d dry period, Constant diet, +BCP, constant diet proportions.

BCP treatment

A sterile, prolonged-release, sponable formulation of a monomeric BSA-derived bovine somatotropin analogue (BST, PCEB-ACW, 300 mg in 1.4 mL, Monomax Bt, Lova, MD) was used for experiments. Injections of BST (10-20 mg BST/mL) began approximately 4 wk (1-2) before expected calving dates. Regardless of time of last injection before calving, first parturition injections were within 30 h of calving and

thereafter injections were at 2 wk intervals. Last injection was at 40 to 60 d. All MCT injections were subcutaneous in the post-scapular region or on either side of the vertebral base. Injections were administered after blood collection, but were prior to a cow leaving its milking. All cows received a full dose of MCT beginning 30 to 40 d postpartum and injections then were continued biweekly during the remainder of their lactation, according to OBG herd practice.

BCP Injections

All 30-d dry cows received a single injection of either 7.5 mL BCP (2 mg BCP/mL; as follows used in, 15 mg BCP; Pharmacia & Upjohn, Kalamazoo, MI) or cotton seed oil (COSO). The BCP and COSO were injected IM at the time they were dried off.

Drying-off Procedure

Cows in both 30-d and 60-d dry groups were completely milked out on the last milking prior to drying off. A syringe containing *Quaternolene®* (penicillin and dihydrostreptomycin, Pharmacia & Upjohn, Kalamazoo, MI) was warmed and the plunger end of syringe inserted into teat canal and other orifices were slowly infused. The same procedure was applied to each quarter. The syringe was discarded after use. Teat discs were taped onto *Breugholite®* (Wor-Aggs, Inc., Kansas City, MO) and sealed to protect against any micro-bacterial entry into the quarters via the teat canal.

Feeding Program

Cows were housed and managed in an open-sided barn stall barn equipped with Calks electronic feeding gates (American Calve Inc., Norwood, MI) beginning approximately 4 weeks before expected calving day. Cows were assigned to the individual gates randomly, then each cow was fitted with a computerized key for her specific gate.

Transponders were placed around the neck of cows so each could open the assigned gate and they were trained to use their gates. In one wk before final intake measurements were recorded. The barn, including feeding area, was covered with a metal roof for protection from rain and sun. Cows were equipped with feed and sprayers which helped keep cows when ambient temperatures increased above 25 °C. Before calving, all cows had access to a hot loading lot where they could calve. Clean fresh water was provided for water troughs for ad libitum consumption. After calving, cows were moved to an adjacent free-stall barn also equipped with gates, feed and sprayers. They remained in this barn for 28 d after which they were moved to the DMR milking herd and fed the same (because TRMR free choice but intake was not measured).

During the time cows were in the free-stall barns, they were fed once daily (08:00-12:00 h) and feed adjustments were made daily. Cows were fed ad libitum to allow 3-10% daily feed refusal. During the first week of the trial, when they were being trained to use Color gates, they were fed a constant CUD diet (1-120 ml/kg BW) DMI ration which was formulated for the average weight of the cows. Starting 3 wk before expected calving date, cows were switched to the constant CUD (1-18 to -13 ml/kg BW) DMI or they remained on the same constant CUD (Table 3-1).

Because feed was offered only one time each day, a wet pushed milk into the Color gate feed was several times daily to ensure that they had access to all their feed. After parturition, all cows were fed a total mixed ration (DMR) based on cows age, whole cotton seeds (WCS), and grain concentrate. This met the requirements of high producing lactating cows (Table 3-2) (NRC 1984, 1993).

Body Condition Score and Body Weight

Body condition scores (3–5, that is fat, Edmonson et al., 1989) and BW of cows were recorded during the experiment. Each cow was weighed and BCS was estimated at 00 and 20 d before expecting calving and weekly on the same day each week (Saturday) before a.m. feeding or milking (07:00 to 11:00 h) through 20 d postpartum. Thereafter BCS and BW were estimated biweekly until they completed the experiment (> 100 d postpartum).

Blood Collection, Handling, and Storage

Blood samples were collected from the tail vein of all cows three times weekly before the a.m. feeding or milking (07:00–10:00 h). Cows were fixed in the free stall barn from the tail vein after elevating the tail without any other restraint. For serum collection, Vacutainer® blood needles (1.14-in, 20 gauge) and tubes containing no anticoagulant were used (10 × 100-mm blood collection tubes, Becton Dickinson, Franklin, NJ). Serum samples were allowed to clot at room temperature for ~1 h after collection, then placed on ice and processed within 2 h. The order in which cows were sampled on a given day was random and differed among days. After sampling, cows were milked and then returned to the free-stall barn.

All blood samples taken to harvest serum were centrifuged at 3000 RPM at 4 °C for 30 min in RC 10 refrigerated centrifuge (Fisher swinging bucket, H-600A rotor, Sorvall Instruments) to separate the serum from the clot. Serum from each sample was aliquoted into 2 labeled 1.5-mL (15 × 2 mm) polypropylene tubes, capped, and frozen at 10 °C until analyzed. The serum samples were used only for the analysis of Ca.

Table 2-1 Dry Matter Concentrations and Chemical Composition of TMR with Whole Corncobs (Lactation Diet) Fed to Holstein Cows During Experiment.¹

Ingredients	TMR
Corn/Silage	22.38
Alfalfa Hay	11.85
Colostrated Hulls	1.82
Corn Pulp	9.94
Haylage	14.38
Duo-Ton/Orena	10.42
Baylean Meal	1.38
Whole Corncobs	50.75
Mixed Mol	1.12
Chemical Composition	Percentage ²
DM	62.10
CP	17.14
Starch/CP ³	31.66
ADF	22.53
NDF	34.66
EE ⁴	7.56
TDN	62.83
NE _L (Mcal/kg)	1.56

¹From NIDRA Forage Laboratory, Ithaca, NY, analysis of ingredients. ²DM basis. ³Percentage of the CP. ⁴Wheat meal.

Determination of Calcium in Serum Samples

Calcium standard solution (1000 ppm Fisher certified) was diluted 10-fold in a volumetric flask to prepare 100 ppm-Ca stock solution. From the stock Ca, 5 and 10 ppm standards were prepared (Table 3-3). To prepare 1% lanthanum (La), 11.73 g Lanthanum Oxide (La_2O_3) was added to a 2 liter pyrex beaker and dissolved in 200 mL concentrated HCl in a fume hood. After the solution released O_2 and Cl_2 and all La was in solution, it was transferred to a 1 L volumetric flask and brought to 1 L with deionized water to prepare a 1% La solution. To prepare a 50% TCA solution 500 g of trichloroacetic

Table 3-3 Standards for calcium determination using Atomic Absorption Spectrophotometer

Ingredient	Standard, ppm		
	0	5	10
100 ppm-Ca stock, mL	0	5	10
50% TCA, mL	10	10	10
1% La, mL	10	10	10
TCA = Trichloroacetic acid, La = Lanthanum			

acid (TCA) was dissolved in deionized water in a 1 L volumetric flask. Then 200 mL 1% La and 200 mL 50% TCA were transferred to a 1 L volumetric flask and 600 mL deionized water were added to give a 1% La, 10% TCA solution.

Five hundred μL of serum samples were pipetted into plastic tubes (Eppendorf Inc. Newton, NJ). To precipitate proteins, 4.5 mL of the 1% La-10% TCA solution was added to each sample. Tubes were vortexed for 10 sec and then centrifuged at 3000 RPM for 10

ness at 4 °C (BC-36 H 6004, force-refrigerated-cooler bags, Servall Instruments). About 4 ml of filtrate were transferred into plastic tubes (Sarstedt Inc., Newton, NJ) and samples were analyzed for concentrations of Ca using a Flame Atomic Absorption

Spectrophotometer (Parker-Donner Model 1000; Miles et al., 2002).

Statistical Analysis

Data from this experiment were analyzed in two sections. The first section included data collected for BW and ECS during the first 8 wk postpartum, and during the first 20 d postpartum for DMG. The second section of analysis included data collected during the first 28 d postpartum for DMG and during 14-wk postpartum for BW and ECS. Data were analyzed as nested designs by least squares analysis of variance procedures of SAS (1987). Proc Mixed procedure of SAS was used to estimate individual daily and/or weekly least squares means for specific variables and treatments (Lowe et al., 2000). Statistical analyses were performed for BW, ECS and DMG. Time periods considered for data analysis were the overall postpartum period (0 to 6 wk), dry and weaning values this period, overall postpartum period (1 to 14 wk) for BW and ECS, and the time period 0–20 d postpartum for DMG. In addition, gross correlations were estimated.

Models included the main effect of lactation treatment (LMT), effect of dry period length (DLY), effect of postpartum diet (DDT), season (SEA, 1—cows with dry periods during hot months [September, October, March, April, and May], 2—cows with dry periods during cool months [November, December, January, and February]), interactions among the treatments and SEA, cow(alt)*DLY*DDT*SEA, and weeks or days to the highest order significant for overall postpartum and postpartum periods.

Results

The recorded data were analysed in two separate data sets. The data obtained during these periods included BW, BCS and DM. The first set included data collected during the final 8-wk preparation for BW and BCS, and during the final 12-d preparation for DM. The second set of analyses included data collected during the final 28-d preparation for DM and 34-wk preparation for BW and BCS.

Changes in Body Weight and Body Condition Scores

Preparation period

Least squares analysis of variance for BW and BCS during the overall preparation period (wk -8 to wk +1) are in Table 3-4. No significant effects of HT, DRY, SEA, or DRYT were detected, however the interaction DRYT*SEA was significant for BW ($P=0.001$) and BCS ($P=0.001$) and the interaction SEA*DRY was significant for BCS ($P=0.003$) but not for BW. There was a significant quadratic effect of WK detected for both BW ($P=0.0001$) and BCS ($P=0.0001$).

During the period from -8 wk preparation to day of calving no differences were detected among the treatment groups for mean BW or BCS. Least squares means for BW and BCS during the preparation period are in Table 3-5. The mean preparation BW of cows in HT exposed and extended cows were 448 and 442 kg, respectively. Both groups of cows gained BW from -8 wk to parturition at -1 wk (Figure 3-1). At -8 wk, the control group cows weighed only 4 kg more than cows exposed with HT. During the final week before calving increases in BW compared to that at -8 wk were 5 and 10% for control and HT exposed groups of cows, respectively. The mean preparation BCS for the same treatment groups also followed the same pattern (Table 3-4, Figure 3-2). No differences

Table 3-4. Least Squares Analysis of Variance for BW and BCS of Holstein Cows During Pregnancy Period (July 18 to Feb 1)

Source	BW				BCS		
	df	MS	F	P < F	MS	F	P < F
AGE ¹	1	4886.12	6.71	0.0098	0.498	0.37	0.5398
SEA ²	1	2488.76	4.32	0.0384	0.226	0.34	0.5632
DAY ³	2	1942.58	1.22	0.2980	0.217	0.11	0.8893
GEST ⁴	1	20184.70	9.84	0.0027	0.276	0.80	0.3709
AGE*GEST	1	724.45	0.40	0.5268	1.122	0.14	0.7182
AGE*SEA	1	8849.08	8.28	0.0026	0.111	0.20	0.6526
AGE*DAY	2	2329.24	0.50	0.5738	0.183	0.10	0.9045
SEA*GEST	1	10912.71	4.95	0.0283	1.211	0.76	0.3811
DAY*GEST	2	2998.24	4.11	0.0123	0.167	1.09	0.2711
AGE*SEA	2	14738.40	1.58	0.2078	1.251	1.40	0.2514
AGE*DAY*SEA	2	1449.21	0.34	0.7087	0.219	0.34	0.7084
AGE*DAY*GEST	2	1401.88	0.28	0.7595	0.182	0.12	0.9014
AGE*SEA*GEST	2	2286.88	0.29	0.9370	0.088	0.11	0.7342
AGE*SEA*DAY	1	2026.89	0.24	0.6242	0.168	0.18	0.7037
AGE*SEA*DAY*GEST	2	9515.44	0.48	0.6344	0.162	0.20	0.7666
Cow (AGE*DAY*GEST*SEA)	44	2420.82	66.47	0.0001	0.117	25.48	0.0001
WE	1	21496.24	491.15	0.0001	1.164	184.11	0.0001
WE*AGE ⁵	1	4282.15	11.08	0.0004	0.261	4.21	0.0409
Error ⁶	100	242.82			0.104		

¹AGE=linear age-at-calving treatment (0=0 to 100, 0=0-1 day (AGE), ²SEA=Season (0=non-lactating periods during last months (December/January/February), 1=lactating periods during last months (March/April/May)), ³DAY=linear age-at-calving treatment during last months (December/January/February), ⁴GEST=linear age-at-calving treatment (0=0 to 100 day period, 1=10 to 100 day period + 100, 2=10 to 100 day period), ⁵WE=Weight (0=0 to 100, 1=100 to 1000), ⁶Error=Error (0=0 to 100, 1=100 to 1000), ⁷Type I Error of Significance for WE, 0.05, otherwise Type III.

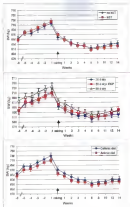


Figure 3-1 Body weight of Holstein cows during the prepartum and postpartum periods. Arrow indicates calving.

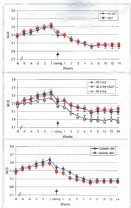


Figure 1-3 Body condition scores of Holstein cows during the prepartum and postpartum periods. Arrow indicates calving.

were observed in mean BCS of untreated and BHT treated cows at 0 wk (1.28 for both groups). The BCS of both groups remained during preparation period and highest BCS was one week before calving (control=3.30 vs. treated=3.33).

The mean BW of cows in 30 d dry period group was significantly greater (678 kg) when these cows were dried off at 0 wk than those of cows in 30 d dry (636 kg) and 30 d dry + BCP (638 kg) groups of cows (Figure 3-4, Table 3-5). However, at the time of 30 d drying off difference was not and not significant (678 kg vs BHT and BCP kg, respectively). Cows in all treatment groups continued to gain BW during weeks following dry off and at 1 wk before calving increases were 19%, 11%, and 10% for cows in 30 d dry, 30 d dry and 30 d dry + BCP groups, respectively (Table 3-5). In relation to BWE, the mean BCS for groups of cows did not differ at 0 week (Table 3-5). The mean BCS of cows in 30 d dry + BCP group was numerically greater (3.33) than those in 30 d dry (3.27) and 60 d dry cows (3.30). Mean BCS increased 3.36, 3.35 and 3.58 points for cows in 30 d dry, 30 d dry and 30 d dry + BCP cows at wk 5 and continued to increase during the preparation period (Figure 3-2). The increase in mean BCS during the last 30 d of the dry period for cows in 30 d dry, 30 d dry + BCP and 60 d dry cows were 0.22, 0.15 and 0.13 points, respectively.

No difference in mean BW for diet treatments were detected (Table 3-4). Cows fed the treated diet had numerically lower BW (648 kg) than cows fed control diet (666 kg) at 0 wk (Table 3-4). Cows in both groups increased their BW during the weeks of the preparation period. The mean increase from wk 0 to wk 1 was 7.5% and 11.3% for untreated and treated diet groups, respectively (Figure 3-3). Similarly, no difference was observed in mean BCS by diet treatment during the preparation periods (Table 3-4). The

mean BCS of cows fed constant and constant diets at wk -11 were 1.36 and 1.38, respectively. Both groups of cows increased their BCS from -1 wk to parturition (Figure 3-2). Increase in mean BCS was numerically but not significantly greater for cows in constant group (3.29 points) than cows in constant diet group (3.22 points) throughout period.

Another objective of the current study was to evaluate the changes in BW and BCS throughout the early postpartum period (wk 1 to wk 14) and to determine if postpartum treatment had effects on postpartum BW or BCS. To evaluate this time period a second series of analyses was performed. Least squares analysis of variance for BW and BCS during the early postpartum period are in Table 3-4. There were no significant effects of DST, DRY, DRET or SGA observed for BW. However, the interaction SGA*DRET ($P=0.041$) was significant. Cows effect of WOC on BW also was significant. There were no significant DST, DRET or SGA effects observed for BCS during the same time period. Significant effect of DRY treatment on postpartum BCS was detected ($P=0.001$). The interaction SGA*DRY ($P=0.000$) and cows effect of WOC also were significant.

The birth weights of calves also were analyzed. The birth weights of calves of cows exposed or not exposed to DRET did not differ significantly (31.3 ± 1.3 vs 30.3 ± 1.3 kg, respectively). The calf birth weights were 31.0 ± 1.4 , 30.6 ± 1.4 and 29.2 ± 1.3 kg for cows in 30 d dry, 38 d dry + BCP and 60 d dry groups, respectively. No differences in birth weights of calves were detected for cows fed postpartum constant (QT1 = 1.4 kg) or constant diets (QT2 = 1.4 kg).

Table 1.1. Least Squares Analysis of Variance for RFP and RCE of Wetland/Grass-Ranging Pastureland
Period July 1 to Feb 1992

Source	RFP				RCE		
	df	MS	F	P<F	MS	F	P<F
INT ^a	1	183.939	9.11	0.0043	0.891	9.95	0.0030
SEA ^b	1	4338.35	1.34	0.2489	0.039	0.04	0.8399
DAY ^c	3	14933.49	4.46	0.0071	1.944	1.87	0.1373
DECT ^d	1	14439.43	1.33	0.2675	0.408	0.46	0.4948
WETFOOT	1	6933.37	0.39	0.5303	1.467	1.73	0.1944
WETSEA	1	540.14	0.01	0.9194	0.013	0.02	0.8854
WETDAY	3	3839.49	0.40	0.7043	0.181	0.04	0.9790
SEA*DECT	1	9540.43	1.19	0.2793	0.036	0.11	0.7381
DAY*DECT	1	10449.34	1.26	0.2643	0.139	0.15	0.6931
DAY*SEA	3	18031.61	0.01	0.9479	0.043	1.13	0.3341
WETDAY*SEA	1	3849.13	0.11	0.7373	0.104	0.26	0.6105
WETDAY*DECT	3	3462.14	0.13	0.9339	0.005	0.04	0.9817
DAY*SEA*DECT	3	14913.95	0.20	0.9794	0.026	0.16	0.8379
WET*SEA*DECT	1	13467.86	0.01	0.9740	0.007	0.01	0.9188
WET*SEA*DAY*DECT	3	35463.35	1.20	0.2846	0.007	0.11	0.7413
Err (WET*DAY*DECT*SEA)	91	39769.47	49.14	0.0001	0.014	14.99	0.0001
Tot	1	67620.46	109.14	0.0001	1.953	186.34	0.0001
WET*SEA	1	10119.48	141.31	0.0001	1.463	72.33	0.0001
WET*SEA*DAY ^e	1	17546.43	19.55	0.0001	0.043	10.35	0.0001
Grand ^f	762	434.73					

^aINT=Random non-spatial error term (0-to INT, 0= 0.0 to 99.999); ^bSEA=Season (0= cool wet dry period during last month (December), October, March, April, and May); ^cDAY=rainfall dry periods during cold months (November/December), January and February); ^dDECT=Days period treatment (0= 00 to 140 period, 10= 30 to 60 period + RCE, 100= 80 to 60 period); ^eWET=Temperature for treatment (0=Temperature Austin, Tex, 10=Temperature Odessa, Texas/7000+work); ^fType I Sum of Squares for RCE alone, others are Type III.

During the postpartum period, mean changes in BW did not differ due to MST treatment (Table 3-4). Mean BW decreased sharply after parturition in both groups. The decrease in BW was observed up to 6 wk postpartum. After this point, cows in both groups maintained their BW (Figure 3-4, Table 3-7). No differences were detected in mean BCS between-MST expected and unexpected cows following parturition. Changes in BCS generally followed the same pattern as BW. The decrease in BCS lasted to 6 wk postpartum and thereafter remained slightly above 2.0 for cows in both treatment groups (Figure 3-2).

Least squares means and SE for BW during the postpartum period (wk 0 through 14) for dry-period treatment are in Table 3-7. Mean BW of cows during the postpartum period did not differ due to dry-period treatment. Cows in all three treatment groups showed an acute decrease in mean BW after calving, as would be expected. Decreases in BW were similar from wk -1 postpartum to wk 6 postpartum when cows stopped losing BW (Figure 3-4). Least in BW for cows in 30 d dry group (23.7%), 30 d dry w/ ECP (14.6%) and 30-d-dry ECP groups (23.1%) were similar from wk -1 through wk 6.

During the postpartum period, changes in mean BCS differed significantly ($P<0.0002$) among dry-period treatments (Table 3-4). Decreases in BCS were detected in all three groups following calving. Changes in BCS were larger for cows in 30 d dry cows and remained significantly less than cows given shorter dry-period after wk 2 postpartum. The BCS reached lowest values around wk 6 postpartum for cows in the 30 d dry groups, whereas cows in 30 d-dry treatment lost BCS up to wk 10 (Figure 3-2). Mean BCS at wk 8 were greater than 3.0 for cows in 30 d dry (3.18) and 30 d dry +ECP (3.17) cows, but less than 3.0 (3.08, Table 3-7) for cows with a 60-d dry period.

100

一	二	三	四	五	六	七	八	九	十
十一	十二	十三	十四	十五	十六	十七	十八	十九	二十
二十一	二十二	二十三	二十四	二十五	二十六	二十七	二十八	二十九	三十
三十一	三十二	三十三	三十四	三十五	三十六	三十七	三十八	三十九	四十
四十一	四十二	四十三	四十四	四十五	四十六	四十七	四十八	四十九	五十
五十一	五十二	五十三	五十四	五十五	五十六	五十七	五十八	五十九	六十
六十一	六十二	六十三	六十四	六十五	六十六	六十七	六十八	六十九	七十
七十一	七十二	七十三	七十四	七十五	七十六	七十七	七十八	七十九	八十
八十一	八十二	八十三	八十四	八十五	八十六	八十七	八十八	八十九	九十
九十一	九十二	九十三	九十四	九十五	九十六	九十七	九十八	九十九	一百

with a single antibody that is not binding to the target. In this case, the antibody is not binding to the target, and the signal is not detected.

TABLE 11. Continued.

	1997		1998		1999		2000		2001		2002		2003		2004		2005	
	500	75	500	75	500	75	500	75	500	75	500	75	500	75	500	75	500	75
WFO-500																		
WFT1	870	± 11	847	± 10	823	± 11	828	± 11	828	± 11	828	± 11	828	± 11	828	± 11	828	± 11
WFT6	887	± 10	864	± 9	845	± 10	823	± 10	823	± 10	823	± 10	823	± 10	823	± 10	823	± 10
WFT7	861	± 9	870	± 11	843	± 9	824	± 9	824	± 9	824	± 9	824	± 9	824	± 9	824	± 9
WFT8	839	± 13	835	± 13	839	± 13	827	± 13	827	± 13	827	± 13	827	± 13	827	± 13	827	± 13
WFT9	834	± 13	833	± 13	823	± 13	824	± 13	824	± 13	824	± 13	824	± 13	824	± 13	824	± 13
WFT10	848	± 11	833	± 10	848	± 10	823	± 10	823	± 10	823	± 10	823	± 10	823	± 10	823	± 10
WFT11	834	± 10	849	± 10	827	± 10	821	± 10	821	± 10	821	± 10	821	± 10	821	± 10	821	± 10
WFO²																		
WFT1	1,481	± 1,046	1,338	± 876	1,361	± 1,050	1,338	± 846	1,338	± 846	1,338	± 846	1,338	± 846	1,338	± 846	1,338	± 846
WFT6	1,391	± 1,111	1,248	± 830	1,404	± 1,021	1,366	± 836	1,366	± 836	1,366	± 836	1,366	± 836	1,366	± 836	1,366	± 836
WFT7	1,524	± 938	1,338	± 859	1,391	± 946	1,338	± 846	1,338	± 846	1,338	± 846	1,338	± 846	1,338	± 846	1,338	± 846
WFT8	1,311	± 1,007	1,117	± 837	1,311	± 846	1,338	± 846	1,338	± 846	1,338	± 846	1,338	± 846	1,338	± 846	1,338	± 846
WFT9	1,388	± 1,027	1,389	± 1,025	1,388	± 1,027	1,366	± 977	1,366	± 977	1,366	± 977	1,366	± 977	1,366	± 977	1,366	± 977
WFT10	1,342	± 1,033	1,386	± 1,044	1,386	± 1,020	1,366	± 928	1,366	± 928	1,366	± 928	1,366	± 928	1,366	± 928	1,366	± 928
WFT11	1,448	± 1,033	1,351	± 878	1,448	± 1,021	1,338	± 846	1,338	± 846	1,338	± 846	1,338	± 846	1,338	± 846	1,338	± 846

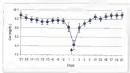
¹ WFO-500 measurements measured globally, 0–500 mg dwt/0.5 m² day per 0.1 increments 0–20 d by year; 0–30 d by year; 0–30 d by period (WFO-500 d by period). WFO-500 values are rounded (0–500 mg dwt/0.5 m² day per 0.1 increments 0–20 d by year; 0–30 d by year; 0–30 d by period).

² WFO-500 values are rounded (0–500 mg dwt/0.5 m² day per 0.1 increments 0–20 d by year; 0–30 d by year; 0–30 d by period).

No differences in mean BW or BCS during the postpartum period (wk1 through 14) were detected between progenitors sown and control diet treatments. Mean BW and BCS for cows in the two progenitor diet treatments followed the same pattern (Figures 3-1 and 3-2). Decreases in BW and BCS were seen through the first 6 wk after calving but after that they increased their mean BW and BCS (Table 3-7).

Serum Calcium Concentrations

Concentrations of Ca in serum did not differ significantly due to tiller, dry period, or progenitor diet treatments. Serum concentrations declined around parturition and were lower the day following calving (Figure 3-3). However, only 16 of 80 cows had serum concentrations of Ca less than 7 mg/dL the day following calving. Concentrations of Ca during the 2 wk before through 2 wk after calving in unexpected cases were 5.4 mg/dL and 5.38 mg/dL for cows expected with HST. On the other hand, 11 of 18 cows that had Ca concentrations intermediate than 7 mg/dL on the day following calving were in the unexpected cases. Overall 1-14 d through 1-14 dL serum concentrations of Ca were numerically greater for 60-day cows (5.96 mg/dL), less for 30-d dry cows (5.57 mg/dL) and least for cows on 90 d dry +ECP (5.28 mg/dL). Five cows in 60 d dry group, 8 cows in 90 d dry no ECP group and 1 cows in 90 d dry ECP group had Ca concentrations less than 7 mg/dL following calving. Feeding the second diet did not significantly improve progenitor or postpartum concentrations of Ca. Cows fed the progenitor sown diet had lower serum concentrations of Ca (5.55 mg/dL) in days 20-cows fed progenitor control diet (5.34 mg/dL), and 5 out of 18 cows fed the control diet had serum concentrations of Ca lower than 7 mg/dL the day following calving. However, no cases of clinical hypocalcemia was observed in any cows irrespective of diet fed.



Changes in Dry Matter Intake

A major objective of this experiment was to evaluate changes in DMI during the lactation period starting 21 d before parturition and continuing through +28 d postpartum. Therefore, data were divided into prepartum (-21 to -1 d) and postpartum (0 to 28 d) sets for analysis. During the prepartum period, no differences were detected in DMI for hMT, hMT or hMT treatments. The two-factor interaction of DSA² × DMT was significant ($P < 0.021$) and main effects of days ($P < 0.001$) and quadratic effects of DCS ($P < 0.0004$) also were significant.

No differences in DMI were detected for hMT treatment groups before parturition. Least squares analysis of variance for DMI in Table 3-4 and least squares means for DMI during the prepartum period are in Table 3-5. Average DMI on 3 wk before calving was greater than 22 kg/d for cows in both hMT treatment groups. Eight days before calving, some decreases in DMI and initial feed intake (DMI) (18 kg/d for both groups; Figure 3-4). From this point, declines in DMI were observed for cows not exposed to hMT and 2 d before calving the DMI for this group was about 1.8 kg, which was a 26% decrease. On the other hand, DMI of cows in hMT treated group had declined to ~12 kg/d, a 13% decrease in DMI. One day before parturition for cows in unsupplemented and hMT treated groups of cows the DMI was reduced to 1.4 kg and 1.5 kg, respectively. Reductions in DMI were 40% for unsupplemented and 37% for hMT treated cows from d. 0 to d. +1 postpartum (Figure 3-4).

Least squares analysis of variance for DMI of the cows in the first dry period treatment group during the overall prepartum period is in Table 3-4. No significant effects of dry period length were detected for DMI. Average DMI 3 wk before calving

Table 1-8. Landowner Analysis of Variables for CDE of Biological Forest Grazing Properties (continued)

Survey	(CDE)			
	df	SS	F	P-Value
SEX ¹	1	199.49	1.19	0.2781
AGE ¹	1	129.13	0.86	0.3595
DEPT ¹	2	162.74	0.26	0.7693
DEPT ²	1	0.04	0.00	0.9513
SEX*DEPT	1	136.25	0.45	0.5050
SEX*AGE	1	191.04	1.13	0.2897
SEX*DEPT	2	49.40	0.27	0.7632
AGE*DEPT	1	166.19 ³	1.03	0.3151
SEX*DEPT	2	14.92	0.10	0.9370
SEX*AGE	2	1.49	0.01	0.9994
SEX*DEPT*AGE	2	10.19	0.16	0.7969
SEX*AGE*DEPT	2	107.32	0.46	0.6389
SEX*AGE*DEPT	2	107.34	0.66	0.5269
SEX*AGE*DEPT	1	107.33	0.42	0.5199
SEX*AGE*DEPT	2	23.34	0.49	0.6161
Gen(SEX*AGE*DEPT*AGE)	20	144.50	10.07	0.0001
SEX	1	141.19	10.08	0.0001
DEPT	1	155.29	11.16	0.0008
AGE	1	168.10	12.43	0.0001
SEX*AGE	1	109.11	10.13	0.0001
SEX*AGE*DEPT	1	171.29	10.14	0.0001
Total ⁴	1289	28.21		

SEX=Sex of landowner's household (1-Male, 2-Female); AGE=Age (years) of landowner; DEPT=Department (1-County, 2-City, 3-Town, 4-Village, 5-City and County, 6-City and County, 7-City and County, 8-City and County, 9-City and County, 10-City and County, 11-City and County, 12-City and County, 13-City and County, 14-City and County, 15-City and County, 16-City and County, 17-City and County, 18-City and County, 19-City and County, 20-City and County); SEX*AGE=Sex and Age interaction; SEX*DEPT=Sex and Department interaction; AGE*DEPT=Age and Department interaction; SEX*AGE*DEPT=Sex, Age, and Department interaction; Gen(SEX*AGE*DEPT*AGE)=Generalized Linear Model (GLM) for SEX, AGE, DEPT, and AGE*DEPT interaction; Total=Total sum of squares for CDE model, which are Type III.

Table 1.3. Linear Equations (Means and SE of Diff., BW and BCS During Pregnancy and Postpartum Periods for Holstein Cows Registered or Not Registered with HST

	Pregnancy (31 to 147)						Postpartum (0 to 180)					
	HST Treatment ^a			HST Treatment ^a			HST Treatment ^a			HST Treatment ^a		
	1	2	3	1	2	3	1	2	3	1	2	3
BW Reg	402.4	± 10.6	444.3	± 11.4	429.8	± 10.1	423.8	± 10.1	423.8	± 10.1	423.8	± 10.1
BCS	3.38	± 0.04	3.43	± 0.05	3.38	± 0.05	3.33	± 0.05	3.33	± 0.05	3.33	± 0.05
Energy Balance (Mcal/d)	—	—	—	—	—	—	16.57	± 0.11	18.20	± 0.11	18.20	± 0.11
Diff. (Mcal/d)	1.80	± 0.03	1.83	± 0.04	1.80	± 0.04	1.80	± 0.04	1.80	± 0.04	1.80	± 0.04
Diff. (kg/d)	22.7	± 0.34	23.4	± 0.36	22.7	± 0.36	22.7	± 0.36	22.7	± 0.36	22.7	± 0.36

^a Treatments include HST- and FreshGuard 20-0-0-2 mg HST/d beginning d-20.

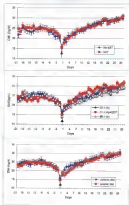


Figure 3-8 Dry matter intake of Holstein cows during the prepartum and postpartum periods. Arrows indicate calving.

was about 23 kg for cows in all dry period treatment groups (Table 3-10). Cows showed no decrease in DM of feed before calving and still had similar DM to that at d 21 (Figure 3-4). However, cows in all dry period treatment groups showed decreases in mean DM during the last few days prepartum with decreases in DM of 11, 37 and 45% for cows at 28 d dry, 28 d dry +ICP and 40 d dry cows, respectively (Figure 3-4).

The least square analysis of variance for DM of cows fed constant and constant diets prepartum did not differ during the prepartum period (Table 3-8). The mean DM were 23.3 kg/d and 23.4 kg/d for cows fed prepartum constant and constant diets, respectively (Table 3-11). Cows in both diet groups maintained their intake through d 8 with DM greater than 23 kg/d. Cows in both treatment groups showed a dramatic decrease 60 day before calving (Figure 3-4). The decrease in mean DM from d -6 to d 1 were similar (37% and 35% for cows fed constant and constant diets, respectively).

Prepartum period

For DM data collected after parturition (0 to 28 d), statistical analyses included the main treatments, their interactions, slope regardless of quadratic order and linear effect of DPM values. During the prepartum period, no differences were detected in mean DM for HSC, DCF or DCT treatment. Significant effects of HSC and the interaction HSC*DMY were detected (Table 3-12). When the DM of cows was expressed as a percentage of BW (DMBW), no effects were detected due to the main treatments HSC and DCT; however, effects due to DMY treatments were detected – significant.

Least square analysis of variance for DM of MT treatment during the prepartum period is in Table 3-13. No differences in mean DM were observed due to HSC treatment. Cows in both MT exposed and unexposed groups showed increased DM

Table 2-20. Listed Exposure Risks and SE of BCR, BCR₂ and BCR₃ During Pregnancy and Postpartum Periods for Mothers Given an Effect-Dose Period Design

	Pregnancy (28 to 36)			
	Dose Period Treatment ^a			
	I	II	III	SE
BW (kg)	477.6 ± 13.0	476.1 ± 13.6	763.1 ± 14.1	
BCR	3.46 ± 0.04	3.43 ± 0.03	3.34 ± 0.03 ^b	
BCR ₂ (Mean)	1.46 ± 0.03	1.38 ± 0.03	1.31 ± 0.03	
BCR ₃ (Mean)	23.8 ± 0.44	22.3 ± 0.43	23.7 ± 0.43	
Postpartum (28 to 36)				
	Dose Period Treatment			
	I	II	III	SE
BW (kg)	414.6 ± 18.43	406.6 ± 19.3	404.1 ± 13.1	
BCR	3.16 ± 0.03	3.19 ± 0.03	2.96 ± 0.03	
BCR ₂ (Mean)	13.77 ± 0.39	15.04 ± 0.43	16.48 ± 1.18	
BCR ₃ (Mean)	1.86 ± 0.04	1.85 ± 0.05	1.73 ± 0.04	
BCR ₃ (SE)	23.1 ± 0.73	22.9 ± 1.05	24.1 ± 0.48	

^a Treatment 1= 28 d effect period with no BCR, Treatment 2= 36 d effect period with BCR₂ and Treatment 3= 36 d effect period

Table 3.11 Least Squares Means and SE of BMC, BCS and BMD During Pregnancy and Postpartum Periods for Holstein Crosses Fed Ascorbic Acid or Control Diet Pregnancy

	Pregnancy (28 to 30)				Postpartum (0 to 30 d)							
	Least Squares Mean		SE		Least Squares Mean		SE					
	1	2	1	2	1	2	1	2				
BMC (kg)	479.5	a	90.71	483.4	a	11.57	421.8	a	9.44	426.0	a	10.45
BCS	3.27	a	0.05	3.43	a	0.05	3.58	a	0.03	3.13	a	0.05
BMD (kg/cm ²)	—	—	—	—	—	—	16.56	a	0.31	17.97	a	0.34
BMD (N/mm ²)	1.24	a	0.02	1.03	a	0.02	1.44	a	0.03	1.63	a	0.02
BMD (kg/cm ²)	26.3	a	0.66	23.4	a	0.48	26.1	a	0.31	31.4	a	0.34

^aTreatment (In Pregnancy Ascorbic Acid, and Postpartum In-Pregnancy Ascorbic Acid), all were observed to occur after pregnancy period.

Table 3.12 Least Squares Analysis of Variance of Yield of Polystyrene-Grafting Polymer Produced (a) 1 to 4 200

Source	df	SS		
		MS	F	Pr > F
LOT ^a	1	11.44	0.08	0.7804
REA ^b	1	1040.09	7.55	0.002
DAY ^c	2	427.40	3.07	0.0287
DAY ^d	1	180.04	1.29	0.2680
LOT*DAY	1	40.90	0.30	0.5840
LOT*REA	1	1848.68	13.39	0.0004
LOT*DAY	2	228.05	1.63	0.0001
REA*DAY	1	329.66	2.39	0.1268
DAY*DAY	2	71.84	0.52	0.5944
DAY*REA	2	343.27	2.45	0.0329
LOT*REA*REA	2	116.05	0.85	0.4303
LOT*DAY*DAY	2	123.76	0.90	0.4092
DAY*REA*DAY	2	481.24	3.49	0.0188
LOT*REA*DAY	1	58.10	0.42	0.5128
LOT*REA*DAY*DAY	2	90.19	0.65	0.5190
Corrected Total (LOT*DAY*DAY*REA)	18	408.26	18.80	0.0004
REP ^e	1	8291.40	59.68	0.0000
DAY ^f	1	14607.70	103.40	0.0000
DAY*DAY	1	3477.48	23.67	0.0000
Total	1999	33.05		

^aLOT=Random monomer/grafting treatment (2=100, 3=150, 4=200 mg/100 g), ^bREA=Reaction time (runs with days) (1=100, 2=200, 3=300, 4=400, 5=500, 6=600, 7=700, 8=800, 9=900, 10=1000, 11=1100, 12=1200, 13=1300, 14=1400, 15=1500, 16=1600, 17=1700, 18=1800, 19=1900, 20=2000, 21=2100, 22=2200, 23=2300, 24=2400, 25=2500, 26=2600, 27=2700, 28=2800, 29=2900, 30=3000, 31=3100, 32=3200, 33=3300, 34=3400, 35=3500, 36=3600, 37=3700, 38=3800, 39=3900, 40=4000, 41=4100, 42=4200, 43=4300, 44=4400, 45=4500, 46=4600, 47=4700, 48=4800, 49=4900, 50=5000, 51=5100, 52=5200, 53=5300, 54=5400, 55=5500, 56=5600, 57=5700, 58=5800, 59=5900, 60=6000, 61=6100, 62=6200, 63=6300, 64=6400, 65=6500, 66=6600, 67=6700, 68=6800, 69=6900, 70=7000, 71=7100, 72=7200, 73=7300, 74=7400, 75=7500, 76=7600, 77=7700, 78=7800, 79=7900, 80=8000, 81=8100, 82=8200, 83=8300, 84=8400, 85=8500, 86=8600, 87=8700, 88=8800, 89=8900, 90=9000, 91=9100, 92=9200, 93=9300, 94=9400, 95=9500, 96=9600, 97=9700, 98=9800, 99=9900, 100=10000, 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1009=100900, 1010=101000, 1011=101100, 1012=101200, 1013=101300, 1014=101400, 1015=101500, 1016=1

after parturition. The first day following calving, cows had their lowest DMI (~ 17 kg/d). Therefore, a gradual increase in daily DMI was observed and one week after calving the DMI of cows in both treatment groups was greater than 23 kg/d, these were similar to greatest intakes seen prepartum (Table 3-1). At the end of the measurement period (d 21) mean DMI for the cows was greater than 30 kg/d (Figure 3-4).

No differences in mean DMI were observed for the three dry period treatment groups after parturition (Table 3-12). However, cows in 60-d dry group tended to have lower mean DMI after calving (Figure 3-4). Cows in the two 30-d dry groups reached DMI that were more prepartum (~ 23 kg/d) 7 d after parturition but 60-d dry cows DMI was ~ 21 kg/d. Dry period periods for mean DMI of 60-d dry cows tended to be less throughout the 28-d period than for cows in the two 30-d dry groups (Table 3-18). The mean prepartum DMI was greatest for cows in 30-d-dry + ECP cows (27 kg/d), lowest for cows in 30-d-dry cows (25.7 kg/d) and least for 60-d dry cows (26.7 kg/d). However, at d 28 postpartum cows in three dry treatment groups, mean DMI was greater than 30 kg/d DMI (Figure 3-4). On the other hand, DMI expressed as a percentage of BW differed among groups ($P < 0.01$). Similar to results for DMI (kg/d), the greatest DMI expressed as a percentage of BW was for cows in 30-d dry ECP group (3.82%), intermediate for cows in 30-d-dry no-ECP (3.66%) and least for cows in 60-d dry group (3.11%), Table 3-19).

The mean DMI during the overall prepartum period (28 d) were similar (24.1 kg/d and 23.5 kg/d, respectively) for cows that calmed and received diets prepartum (Table 3-11). Cows that calmed that tended to have slightly numerically greater DMI during the first week following parturition. However, the DMI of cows in both diet groups was greater than 23 kg/d 7 d postpartum (Figure 3-4).

Energy status of the cows during the first 4 wk postpartum was negative and did not differ significantly for hCT and non-hCT groups (-18.23 vs. -16.67 Mcal/d, respectively; Table 3-9). The energy status of cows fed ad libitum or reduced diets postpartum did not differ during the first 4 wk postpartum (-18.36 vs. -17.97 Mcal/d, respectively; Table 3-10). Cows in 30 d dry group had significantly less negative energy status (-13.77 Mcal/d) than cows in 30 d dry + hCT group (-17.26 Mcal/d) or than cows in 60 d dry group (-20.48 Mcal/d, $P < 0.05$, Table 3-11) during the first 4 wk postpartum.

Regression analyses were performed for DMI to describe trends in consumption throughout the transition period (-25 to 25 d). Regression curves indicated that no differences were observed between hCT injected or noninjected cows as well as between postpartum reduced diet and ad libitum diet treatments. On the other hand, 30 d dry groups appeared to have greater DMI at the beginning of the lactation. Heterogeneity tests of the prepartum and postpartum curves for dry period treatments provided no evidence that curves were not parallel. Results presented in Figure 3-5 showed almost identical trends for hCT injected and noninjected control cows from the beginning of the trial through 25 d postpartum. Cows in postpartum ad libitum diet treatment tended to start with greater DMI at the beginning of the experiment, but no differences were observed during the first weeks postpartum. In addition, cows in postpartum reduced diet treatment also started with greater DMI during the first week following parturition, but DMI of cows in postpartum reduced diet treatment tended to be greater at the end of the experiment. Heterogeneity tests of the prepartum curves provided no evidence that curves were not parallel and all cows in dry period groups had similar trends during the prepartum period.

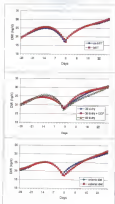


Figure 3.5 Regression-fitting changes in Day water intake of Holstein cows during the prepartum and postpartum periods

Discussion

Postpartum Period

High yielding dairy cows undergo a rapid and extensive metabolic challenge between late pregnancy and early lactation. A significant fall in DMF occurs during late pregnancy and it continues through early lactation (Barton et al., 1992; Cripps et al., 1992; Lodge et al., 1993; Barton et al., 1995) describing two pronounced phases that can affect some health and lactational performance. The first phase takes place during the last week prepartum and is characterized by a 30% or greater decrease in DMF. The second phase takes place during the first 3 wk postpartum, a time when DMF should increase rapidly to support milk production. Rate of increase in DMF is the primary determinant of energy intake and energy balance during early lactation, even and it is important that it occurs during this early postpartum period.

In the current study, limited decline in DMF was observed in all treatment groups before calving. At the end of the Calve gas-measuring period (~ 21 d) cows in all treatment groups had essentially returned their precalve DMF and it remained high for 2 wk except for the cows in 30 d dry ECP group. These cows showed a decline in DMF ~ 13 d which lasted about 1 wk. However, cows in all treatment groups had mean DMF greater than 22 kg/d 1 wk before calving. Decrease in mean DMF from d 14 to d 2 was about 10 % across all treatment groups and overall decrease over d -1 was about 30%. Thus, even though a precalve decline was seen during the last week prepartum, the major decrease in feed consumption took place just before or on the day of calving. The DMF of cows was much greater than previously reported. Average DMF reported 1 wk before calving was around 18 kg/d for Holstein cows (Barton et al., 1992; Garvin et al., 2000; Crain et al.

1996, Lodge et al., 1995, Zarek et al., 1979). They also described a 20 to 40 % decrease in DMG during the final week prepartum. Although our data confirmed previous reports that DMG decreased during the final week prepartum, the decrease in DMG was less than that reported by others (Metcalf et al., 1992, Garcia et al., 2008, Grew et al., 1996, Zarek et al., 1979), but agreed with Galey (1994). In the latter study, decreases in DMG for cows treated with MST (5, 10.2 or 15.3 mg/MST/d) and unsprayed control cows were between 17 to 23% at 1 wk before calving compared to that at wk -3 before calving (Galey, 1994). On the other hand, Garcia (2008) reported that there was about 14 % decrease in mean DMG 1 wk before calving for cows sprayed and unsprayed with MST. On the day before calving the DMG of both sprayed or unsprayed groups of cows was about 63 % of that seen on d-7 (Garcia, 1998).

Greater decreases in DMG for cows sprayed with MST (5 and 15 mg/d) was reported before calving (Barnum et al., 1994). Zarek et al. (1979) reported that DMG decrease during the last week before parturition was associated with high incidence of metabolic diseases during early lactation, which differed from the current study. During the present study, no clinical hypocalcaemia (milk fever) was observed. Only two cows post calving from all 4-day, no-MST, treated and group and one cow from all 4-day MST treated that group presented displacement of abomasum (DAO). The EO-remission rate for this experiment was about 2.4% which was much less than the average observed in the DMG herd and others reported for cows up to 14% (Duckley, 1999, Fox and Graham, 1993).

In current experiment, the relatively high DMG during the prepartum period also affected the BW and BCS for the cows and from wk -4 up to wk -1. The BW and BCS at

the cows increased on a week to week basis, and it was not affected by the duration of DM during the final week preparation. Body Weight and BCS were positively correlated ($P=0.001$, $r=0.676$) during the preparation period. In addition, DM was correlated positively with BW ($P=0.005$, $r=0.646$) but not with BCS ($P=0.240$, $r=0.323$). No differences in DM were observed due to any of the treatments. Fiksel et al. (1995) reported no significant differences in preparation BCS between control cows and those injected with a full dose of MT (380 mg MT/d) when separation began. In a preparation. Similarly, no effects of low doses of MT injected prepartum (2.5, 10.0 or 15.0 mg/d) were observed on BW and BCS compared to control cows (Dwyer, 1998). Garber (1992) also reported no effect of low MT dose injection on BW and BCS (0.5 mg MT/d). Our results indicated that cows in both injected control and MT injected groups had similar DM during the overall preparation period which led to similar BW and BCS trends for both treatment groups. If a difference exists, perhaps a 2 wk time period was too short to detect a difference in DM due to MT, if any effects were provoked during preparation period.

In the current experiment, there was a decrease in mean DM beginning around 21 d which lasted about a week in cows that had been injected with ECP and given a 30 d dry period. Bolander (2003) reported that separating dry cows with ECP resulted in increases in plasma concentrations of E_2 compared to control cows and that peak concentrations of E_2 were reached 48 h post separation and they persisted for up to 14 d. In cows, extensive rejection of ITB resulted in acute decreases in feed intake (Grossowder et al., 1990). Studies in non-reproductive animals has negative effects upon feed intake due to direct actions on the brain (Fiksel et al., 1995). In addition, Dwyer (1998) also

reported depressed feed intake in maternal male sheep when intra-uterine injections of estradiol benzoate greater than 40 µg were given. Oestrogenicity resulted in a temporary increase in feed intake for 3 to 4 wk and then resulted in a decrease in BW in rams (Turbellin and Garcia, 1973). Injection of physiological doses of oestrogen resulted that affect male reduction of BW was observed as long as oestrogen treatment continued (Turbellin and Conks, 1973). Moreover, in ewes, intrauterine injections of E_2 decreased both BW and DM (Graham et al., 1980). Thus, the expected additional treatment in placenta administration of nitrogen due to ECP injections may have had an acute but short-term negative effect on DM, as seen in ECP injected ewes (Schelling, 2007).

No difference in BW or BCS was observed due to dry period treatment. Ewes in all three dry period treatment groups showed increased BW and BCS from wk -4 to wk -1, which continued through the first week lactation. Although ewes in both 30 d dry ewe and maternal ECP were not dried off earlier -4 wk to -1 wk (same period), they gained about the same BCS (0.1 point) as 60 d dry ewes (0.06-point) which were not being milked during this time. Ewes in 60 d dry group had significantly higher BW at the time of drying off (-1wk) than ewes in 30 d dry groups. However, although BW still was numerically greater during the remaining preparation period, no significant differences in BW were observed between 60 d dry and both groups of 30 d dry ewes.

No significant differences in mean DM were detected between preparation diet treatment groups. Ewes in maternal and maternal diet treatments showed gradual decline in DM during the last week preparation with the major decline in mean DM occurring within 24 h of parturition for ewes in both groups. No effect of preparation diet was detected for preparation BW and BCS in current study. The results of current study agreed

with others (Block, 1984; Moore et al., 2000; Orsini et al., 1991). Moore et al. (2000) reported no difference in proportion DMG between cows fed concentrate and pasture diets, in the same study, animals with treatment did not change BW and BCS compared to cows fed the control diet that did not contain silage. In another study, animals with treatment did not cause a decrease in DMG compared to cows fed the control diet without silage, and they reported no difference in proportion BCS of cows fed the different diets (Orsini et al., 1991). Block (1984) also failed to detect a difference in DMG of cows fed concentrate or pasture diets. On the other hand, Vagnoni and Orsini (1994) reported that when diets were fed for two week periods that the daily DMG increased the rate of accretion in the uterus that occurred during the first 3 d. However, mean DMG over d 3 to 7 was reduced when dietary source diets were included due to a mild metabolic acidosis. Because feeding periods were relatively short at their trial, no differences could have been detected if reduced DMG occurred or persisted for a greater length of time. Results of the current experiment indicated that DMG was maintained at a high level in both pasture and concentrate diet groups of cows such that there was no effect on BW and BCS proportion and no negative effects of feeding diet that contained pasture diets were evident.

Early studies demonstrated that a major decline in DMG is obtained in late pregnancy irrespective of diet fed and extending early lactation (Dewdney, 1979; Ingemansson and Andersson, 2000). It has been suggested that the decline in the DMG during late pregnancy period is caused by the pressure on the uterus exerted by the growing fetus and also by the increasing accumulation of abdominal fat during late pregnancy (Parfeni, 1964). However, decreased rumen volume actually can be balanced by an increase in the passage rate of particles out of the rumen (Kotter and Clark, 1997). As a

much, it is most likely that decreased DMI during the late prepartum period is due to metabolic adaptations that occur before calving. It has been proposed that increased accumulation of lipids in the body reserves during the late prepartum period down-regulates DMI (Boman and Donner, 1984).

Increased glucose uptake by the growing fetus causes milk to be consumed exclusively for the use of glucose by maternal tissues. Thus, maternal tissues rely on metabolism of NEFA and ketone bodies (Bell, 1993). Increased lipid stores results in mobilization of body fat and a rise in plasma concentrations of NEFA, glycerol and ketonebodies. It has been speculated that mobilization of NEFA in the brain and the liver can decrease DMI (Chen et al., 1995). In dairy cows, a 4 h release of lipids has provided 11.7 MJ of NE_o, resulted in a slight increase in DMI postpartum (Bomblis and Fawcett, 1986). Moreover, Chen et al. (1997) observed a substantial decrease in DMI during the first 4 h postpartum after they fasted Holstein Friesian and crossbred in dairy heifers by using sodium nitroprusside. It was suggested that the increased plasma glycerol also may influence intake through a central nervous system mechanism. Intracerebroventricular infusion of glycerol is not decreased than feed intake (Boman et al., 1981).

Along with mobilization in lipid, it has been suggested that hormones also affect feed intake. Metabolic adaptations that affect feed intake are regulated, in part, by IGF. Thus, reduced uptake and conversion of lipids leads to decrease in de novo synthesis of TG, and increased lipolysis from body results from decreased activity of IGF to promote lipogenesis and suppress lipolysis (Bell, 1993). Moreover, administration of estrogen may be maladaptation and peak plasma prolactin (Chen et al., 1977). The increased concentrations of TG in the peripheral circulation may have direct effects in the

post-parturient reduction of the hypophyseal (Peters and Reikardt, 1985) and this has been implicated to reduce lipid failure calving (Peters, 1987). In addition to E_{L_1} , concentrations of ST begin to increase during late pregnancy. Somatotropin reduces the ability of OMS to stimulate lipogenesis in adipose tissue and actively stimulates lipolysis. ST also the sensitivity of adipose tissue to β -adrenergic agents (Barnes and Vernon, 1983). These effects would dramatically increase mobilized lipids from the adipose tissue and increase concentrations of NEFA and glycerol in blood. Thus, there would be greatly reduced fatty acid synthesis or, at least, no net synthesis, and hence less acetate and glycerol use as adipose tissue (Barnes et al., 1984). For these reasons, ST is considered the major regulator of metabolic adaptation that occurs during the transition period, or rather, and continues throughout the lactation (McLuskey, 1985).

Inspection Period

Rate of increase in OMS during early lactation is the primary determinant of energy balance along with the rate of increase in milk yield. Thus, the first 3-40 postpartum is important because OMS should increase rapidly to provide the energy and precursors to support and sustain milk production. The current study focused on differences in rates OMS during the first 314 postpartum due to MCT treatment. Cows in both groups showed increased OMS after parturition (Figure 3-4). The first day following calving, cows had the lowest OMS (-17 kg/d). Therefore, rate of increase in OMS for cows in both MCT treated and untreated control groups were similar. At the end of the sampling period (d 314), mean OMS for the cows was greater than 30 kg/d. Also no differences in BW or BCS were seen due to MCT effects. This agreed with results of others where low, intermediate or high doses of MCT had been applied postpartum.

Potmann et al. (1993) reported no significant differences in postpartum BCS of cows in control or prepartum MST-treated groups (300 mg/14 d). In the same study, the DMG was significantly greater at 28 d after parturition. Others also reported no increases in DMG or efficiency of milk production by cows treated with MST during early lactation (Gale and Block, 1990; Schneider et al., 1993). In addition, postpartum DMG of cows treated with lower doses of MST (3 and 14 mg MST/d) during the prepartum period did not differ (Giamberini et al., 1994). Rypard et al. (1996) reported no differences in DMG, BCS or BW during the first 9 wk of lactation of cows treated with full dose of MST (300 mg/14 d) prepartum compared to unsupplemented control cows. When cows were supplemented with 20 d mg MST (gle Boer et al., 1991) or 5 and 14 mg MST/d (Skarzynski et al., 1992), postpartum DMG, BW (gle Boer et al., 1991) or BCS (Skarzynski et al., 1992) were not affected by MST treatment. Modellen et al. (2000) concluded that MST injected very early in lactation (i.e. 10) increased DMG after parturition were initiated. On the other hand, increase in DMG was not enough to support the increase in BW and injected cows lived an equivalent period of MLR and, therefore, BW and BCS decreased. Furthermore, in that study cows received a full dose of MST (300 mg/14d) which resulted a narrow NGB for the injected cows. Additional reports also suggested an increase in DMG of cows when MST was injected during mid to late lactation (Lucy et al., 1993; Ransom et al., 1993). On the other hand, prepartum and postpartum injections of 15.5 mg MST/d increased DMG after parturition, and there was less decrease in BCS and BW allowing the cows to maintain BW and BCS at low rates during early lactation (Lucy et al., 2000). In addition, Giamberini et al. (2000) reported that a low dose of MST (3-1 mg/d) injected nine times before and after

parturition increased maximal DMI during the early weeks of the lactation period, however this was not significant.

In current study, no direct NER was seen, as indicated by the changes in BW and BCS even though MT increased. Good BCS at calving is important for high-producing cows and dry cows should achieve an adequate BCS (>2.5 – 3) at calving (Wood et al., 1992). In current study, cows in MT expected and unexpected groups had BCS of ~ 3.50 at calving and also had high DMI. These two factors might have enabled cows to recover their BCS earlier during postpartum period which would agree with the report that cows having BCS of 3 to 3.5 had better recovery of BCS at 18 wk postpartum than cows with BCS of 4 (Peters et al., 1994). Importantly, low dose exposure of MT during prepartum and early postpartum periods did not have a negative effect on BW or BCS, although the MT expected group was producing greater quantities of milk suggesting that was more efficient production of milk and 3.5% FCM. Thus, the low dose of MT during the treatment period caused less problems for the cows compared to full dose of MT because higher doses resulted in lower NERs and BCS loss for these cows.

In the current study, it is important to note that no negative or positive effects of MT on BW or BCS were observed any time postpartum. Previous reports where full dose of MT following parturition suggested that the effect of treatment on BCS was severe and cows were affected adversely compared to untreated cows (Wood et al., 1994; Mullins et al., 2000). In another study, where full dose of MT (expectant) was given 28 d prior to expected calving date and were continued until parturition, cows treated with MT produced 3.3 kg/l more milk than untreated controls during the first 42 d of lactation (Peters et al., 1994). However, cows in MT treated group had significantly

higher serum BCS that coincide when they were assigned to the trial, these values were confident evidence of expecting a fall since BCT had a negative effect on subsequent BCS. In the current study, overall mean BCS was maintained greater than 3.0 for cows in both BCT exposed and unexposed groups and no differences in DMI, BW and BCS were observed between treatment groups.

No difference in mean DMI or BW were observed due to dry period treatment after parturition. Following parturition, mean DMI increased for cows in all treatments (50 vs. 60 d dry), even though there was a slight seasonal increase in DMI for 60 d dry cows compared to 60 d dry. Although mean BW were similar, cows in 60 d dry period tended to lose more BW after parturition. Cows in 60 d dry group gained about 60 kg during the dry period. Although cows in 30 d dry group gained about 70 kg BW during water time, only ~30 kg of the 70 kg was gained during the dry period, with the remaining 40 kg gained during the milking 30 d they still were milking. Perhaps the greater increase (40 kg) in BW during dry period depended DMI postpartum. A positive relationship has been shown previously between weight gain during dry period and the extent of postpartum maintenance of body tissues (Ragland and Anderson, 2000).

When DMI was expressed as a percentage of BW, it was significantly greater for 30 d dry cows with or without BCT than 60 d dry cows. Furthermore, the mean BCS of the cows in 60 d dry group was significantly less than cows in both 30 d dry groups during the weeks following parturition and they also lost more BCS for a longer time period (Figure 3-3). The average BCS gain for 30 d dry cows and 60 d dry cows was -0.27 and 0.30 points, respectively during the postpartum period. However, although 60 d dry cows were not being milked from wk. 0 to wk. -6, they eventually did not gain more

compared to those 30-d dry cows that still were milking (3.86 vs 0.16 percent, respectively). Apparently, the 60-d dry cows consumed less DM during FOD period because BCS and BW did not increase and yet they were not using substrate to produce milk, as were the 30-d dry cows.

It has been concluded that body reserves replenished during late lactation recover more efficiently than that replenished during the dry period (Mott et al., 1971). Armstrong and Miner (1981) suggested that efficiency of lipogenesis during the ongoing lactation was higher than recovery during the dry period. If BCS increased during late lactation, the efficiency of replacing BCS was ~54%, however, if replaced during the dry period, the efficiency of replacing BCS was only ~17% (Mott et al., 1971). Thus, they concluded that BCS lost during early lactation could be replaced more efficiently during late lactation than during the dry period. The data indicated that 70-d dry period with or without ECP exposure did not have a negative effect on postpartum BCS or lactational performance in the current experiment. On the contrary, results suggested better maintenance of BCS with 30-d dry period than 60-d dry period because BCS of the cows in shorter dry periods were greater ($P < 0.05$) than BCS of cows given the longer dry period ($P < 0.05$). As a result, more persistent BCS was seen in 30-d dry cows, because short-term dry cows gained more of their BCS when dry were still lactating. Moreover, the greatest DM, expressed as a percentage of BW was for cows in 30-d dry ECP group, intermediate for cows on 30-d dry no ECP, and lowest for cows on 60-d dry group. Thus, repeating postpartum ECP did not reduce DM.

Cows in 30-d dry groups experienced only one dry day during their dry period whereas dry for 60 dry cows changed two times. Making changes in dry might ultimately

affect development of papillae and subsequent adaptation of the microbial population of the rumen. Changes in the numbers of rumen papillae occurs in response to nutritional changes. Complete adaptation requires a period of 2 to 3 wk (DeNobels et al. 1981; Goff and Hoot, 1993). Making slower changes in the other feed might encourage maintenance of the desired rumen population and faster rumen papillae development. Thus, it is possible that cows given shorter dry periods would have better efficiency of fermentation and absorption of nutrients due to fermentation during early lactation even though residual DM did not increase linearly or to much greater amounts, yet the changes in fermentation and absorption would result in greater maintenance of RCH during early lactation as was found.

Although mean DM of cows fed organic and inorganic distillers grains did not differ during the first week postpartum, cows fed the organic dist preparation tended to have slightly higher DM following parturition. Urine of the animals was collected and pH measured routinely during the preparation period. Cows in organic dist group did have a lower range of urine pH than cows in inorganic group (5.4 to 6.0 vs 7.0 to 7.6). Typically, more fed animals have lower rumen pH and this causes a mild ruminal acidosis (Hobbs et al., 2006; Gerdul et al., 2007). It is likely that in current study, organic dist resulted in decreased rumen pH during the preparation period. Lower pH in the rumen has been reported to decrease rate of fiber digestion and organic fiber effect of the diet, which might increase retention in the reticulo-rumen (Allen and Martin, 1984). Consequently, it is possible that lower rumen pH immediately after calving due to preparation organic dist might have influenced the DM of the cows that were fed the inorganic dist preparation and caused the slightly lower increase in DM for the cows on the

group. However, the differences in DM for the cows in the two diet groups were small and remained similar during the lactation period.

MCN levels in a common metabolic disorder in dairy cattle and the incidence rate of milk fever among lactating Friesian-type cows is less than 1% (Bull and Garber, 1982). Manipulating the DC:ND before and by providing various rates of propionic acids has been suggested to improve Ca metabolism by increasing intestinal absorption of dietary Ca and/or by increasing bone Ca mobilization (Block, 1984). Cows fed anionic diets tend to have greater circulating concentrations of Ca than cows fed cationic diets prior to calving (Owen, 1988; Goff et al., 1994). However, in the current studies efficient serum Ca was detected due to preparation feeding of an anionic diet. More recently, dietary K also has been shown to have a role in the incidence of milk fever (Goff et al., 1995). Cows with a high K content can increase the risk of hypocalcaemia more than high dietary Ca during the last weeks of the prepartum period. For example, increasing dietary K from 1.15% to 2.1% increased the incidence of milk fever from 40.5 to 50% in lactating cows. Addition of strong cations to prepartum diets causes a metabolic alkalosis and that suggests that bone resorption of Ca is inhibited in cows fed high K or Na diets because feeding these diets results in increased blood and urine pH (Goff et al., 1995; Rossi et al., 1994). In the current study, dietary K was substituted for 1.02% of the DM for the control and 1.14% of the DM for the anionic diet. These low quantities of K in the diet fed to goats have helped some fed cationic diet group to maintain serum concentrations of Ca at an acceptable level and reduced the risk of hypocalcaemia (milk fever).

High producing dairy cows are unable to consume sufficient amounts of energy during early lactation because LFP usually peaks between 3 to 7 wk postpartum, while

maximum DMG is reached between 8 to 22 wk after calving (Ingvarsson and Anderson, 2000). Cows often need to replenish their body reserves during the dry period. Thus, good body-condition (2.25 to 3.75) at calving, is important to high producing cows (Plouffe et al., 1983). On the other hand, overconditioning is not needed and should not occur during the dry period. In the current study, BCS of cows in all treatment groups were at acceptable levels (3.44 \pm 3.09) at calving and postpartum DMG was correlated negatively with BCS ($P=0.004$, $r=-0.674$). It was reported that postcalving, cows with higher BCS lose more reserves than cows with lower BCS and they reach positive energy balance faster (Garnsworthy, 1998). Increased accumulation of lipids in body reserves during the postpartum period down-regulates DMG (Brewer and Brewer, 1999). Moreover, losing weight during the dry period increases incidence of metabolic diseases (Kendall and Clark, 1994). There was a positive relationship between postpartum weight gain and the extent of postpartum mobilization of body tissue (Ingvarsson et al., 1997). If BW gain was excessive 42 kg during the dry period, that caused a depressed feed intake postpartum and caused excessive mobilization of body tissue (Ingvarsson and Anderson, 2000). Furthermore, cows with higher BCS at calving lost weight longer after calving than cows with moderate BCS (Rutgg and Milow, 1995).

In the current experiment, there was a high positive correlation between BW and BCS during the postpartum period ($P=0.00$, $r=0.717$). This supports the view that BCS is indeed a practical, if not quantitative, method of evaluating nutritional status and energy reserves of cows (Rutgg and Milow, 1995). The rate of BCS loss was greatest during the first 2 wk postpartum for cows in all treatment groups and the difference in BCS (from -0.15 to -0.25 points) reflected rapid tissue mobilization that occurred after

calfing. Cows reached their maximum BCS by about 40 to 45 DIM. These results were similar to those reported earlier. Energy and Milson (1993) described a GLD period just at BCS during the first week after calving. Pedroni et al. (1993) reported that maturity of cows that had BCS of 3.0 to 3.5 at calving started at 18 wk postpartum. Moreover, cows with higher BCS at calving (>3.5) appeared to lose BCS for a longer period of time (>60 DIM) than cows with lower BCS at calving (<3.25, 50 DIM) (Garrowood and Jones, 1987). Energy balance is most negative during wk 1 postpartum (Harrison et al., 1986). Thus, the BCS loss in early lactation appears to be rapid. As mentioned above, continued BCS loss from 18 to 90 d was reported (Energy and Milson, 1993; Pedroni et al., 1993). The data from current study showed gradual BCS loss during the first 2 wk postpartum, and cows reached their maximum BCS by 6-8 wk postpartum. Thus, no negative effect of parturition or postpartum treatments of IVE, dry period length or parturition date treatment were observed. The observed the rapid increase in postpartum DIM decreased the rate of postpartum loss in BCS.

Conclusions

Results of this study indicated that use of low doses of IVE during parturition period (0-25 or 3 d) caused no negative or positive effect on the treated-cows. Cows treated or not treated with IVE appeared equally capable of replenishing their body reserves during early postpartum period. Injections of low doses of IVE during the early lactation (0-1 or 3 d) did not have a negative or positive effect on rate of increase in DIM. In addition, cows had the same rate of mobilization of body tissues and loss of BCS irrespective of parturition or postpartum treatment.

No effects of proportion diet treatments were observed on either proportion or postpartum DMG, BW or RCS. No clinical/physiological was observed neither feed and water consumption of CS before and after calving did not differ between feed access and restrict diets. Thus, there were no obvious advantages to support view that restrict diets should be fed to cows during the close-up dry period or compared to a properly formulated constant diet when pregnancy is diet was regulated. Serum concentrations of CEA in Holstein cows during the current trial and diet fed proportion had little or no impact or positive effects on postpartum DMG.

Dry period length did not have a significant effect on DMG, BW or RCS of the cows postpartum. Cows in the shorter dry period groups were able to replace their BW and RCS as well as cows in the longer dry period group. Short dry period cows (< 30 -d) gained more of their BW and RCS while they still were lactating, whereas 60-d dry cows nearly gained BW and RCS after drying off. During postpartum period, short dry period cows lost less RCS postpartum than 60-d dry cows and had more DMG as % BW. Thus, it might be advantageous to allow cows to replace their RCS before they are dried off.

In conclusion, it appears that restriction of NUT during the prepartum and postpartum periods, reduce loss during prepartum period, or shorter (< 30 -d) dry periods feeding requires indication that causal-physiological effects on DMG, BW or RCS changes or health problems during postpartum period.

CHAPTER 4 IMPROVING MILK PRODUCTION AND HEALTH OF COWS BY SHORTENING THE DRY PERIOD WITH NITROGEN AND USE OF MT DURING THE TRANSITION PERIOD

Introduction

Dry Period

The lactation cycle begins with a period of mammary gland development followed by lactogenesis. Milk synthesis and secretion occurs after parturition. After the peak in milk production, a declining phase follows until milk removal is stopped either at weaning or due to the management practices followed by the dairy producer. The lactation cycle of the female continues several times during her reproductive life (Hartley, 1985). The period between weaning or lactation-after period when milk removal is stopped is called the dry period. This dry period allows a remodeling of mammary tissue and the restoration of lactation at a maximal level after next calving (Hartley, 1985).

Many factors must be considered to determine the appropriate length of the dry period for individual cows. In a genetic and environmental study, Schaeffer and Rindfleisch (1977) examined the lactation records of Holstein cows in the New York area. They concluded that age and month of calving significantly affected length of dry period. O'Connor and O'Connor (1988) included parity, month of calving, and time of conception as factors that affect optimum time of drying off. Dean and Allum (1982), after studying nine farms from 1967 Holstein cows, reported that the length of time required for dry period decreased as the lactation number increased from first through fourth lactations. They

stated that cows with calving intervals greater than 145 d required fewer days dry than cows with shorter calving intervals except those in first lactation. Effect of dry period length on milk yield was greater for younger cows and the optimal number of days dry declined from 43 to 23 d as age at calving increased from 29 to 33 mo in the mature study. They also stated that calving interval and daily MY at 150 d before calving were significant factors affecting total days needed for the dry period.

Establishing optimum length of the dry period is critical to achieve maximum milk production during the next lactation. Since HCs may observe actual and experimental data have been generated to establish an optimal drying-off time for cows. Filly (1976) 60 d dry period length has been recommended based on the fact that this would maximize production in the following lactation (Cappuccin et al., 1979; Chen and Adams, 1982; Klein and Woodward, 1983; Schaeffer and Henderson, 1972). Arnold and Bealke (1986) evaluated Jersey cows with dry periods of 35 d or less (10 cows), 31 to 50 d (24 cows), 51 to 60 d (20 cows) and 61 d or more (24 cows) preceding the lactation. In their study a dry period of 31 to 50 d allowed the maximum MY in the subsequent lactation. Klein and Woodward (1983) compiled 11,077 lactation records from Dairy Herd Improvement Association (DHIA). They found optimum dry period was 35 d for cows yielding >3000 kg of 4% FCM with 12 mo calving interval.

Smith et al. (1987) evaluated the effect of milking throughout pregnancy in terms of total milking compared to a dry period of 3 to 9 wk on the effects on milk yield versus the same cows. Two quarters of the udder of 3 cows were milked continuously while the other two quarters were dried-off for > 60 d before expected calving. The quarters allowed a dry period of 3 to 9 wk produced 40% more milk in the subsequent lactation.

Lawrence (1967) took another approach and used first years of seasonal cross dairy cows to evaluate the need for the dry period. One of each pair of seasonal cows was dried off to give a least cost wet dry period, whereas other pairs were milked continuously for two consecutive lactations. Average milk yield of the continuously milked cows in the second and third lactations was 71 and 67% of the control cows that had ~55 d dry period. Results of both studies indicated that the secondary gland benefited from a dry period.

To evaluate the effects of dry period length on late milk production, Coughlin et al. (1974) conducted a 42 mo field trial. Cows ($n=1565$) were assigned to treatments of 20, 30, 40, 50 and 60 d dry periods. Dry period lengths were allowed to have a 10 d range in each group. 382 cows completed the 42 mo study. Cows that averaged less than a 40 d dry period produced 458 to 680 kg less milk in the subsequent lactation compared to cows having dry periods of 40 d or longer. Cows with 40 d dry period produced as much milk as cows in 50 d dry period. When cows in shorter dry period groups were allowed to have longer dry periods during the next lactation, no carry over effect was observed.

Schaffler and Henderson (1972), in a survey based analysis, reported that cows with dry periods of 50-59 d had the highest production in the subsequent lactation. Yet, average milk production for 40 to 49 and 50 to 59 d dry periods did not differ significantly. In another study, Park et al. (1977) analyzed data collected for 8 yr from over 14,000 cows. They concluded that cows given dry periods longer than 70 d produced only moderately less than cows dry for 60-69 d. Moreover, cows dry for 40 d or less produced significantly less (-420 kg) in the subsequent lactation than cows dry for 60 to 69 d which produced the most milk.

In a more recent study, Sorensen and Ewoldsen (1991) evaluated the effect of different dry periods on subsequent milk yield. Cows were dried off at 4, 7 or 10 wk before expected calving. They found a decrease in yield of $2.3 \text{ kg (176 L/CM)}^3$ when dry period length was decreased from 7 to 4 wk, whereas there was a 4.4 kg/l increase in milk production when dry period was increased from 7 to 10 wk. In contrast to the study by Dettl and Allen (1982), Sorensen and Ewoldsen (1991) failed to detect an interaction between the dry period length and lactation number. In another study, effects of days dry on milk yields of Holsteins from Kentucky and North Carolina were evaluated (Molenaar and Molenaar, 1986). In this study first ($n=1144$), second ($n=714$) and third ($n=602$) lactations for cows were evaluated. In both locations shorter dry periods had detrimental effects on milk yield. Milk yields of cows dry for 36-39, 40-49 and 50-59 d were 415, 433 and 282 kg/low than for 60-d dry periods. On the other hand, little advantage was observed for dry periods longer than 60-d.

The dry period length is very important because it is directly related to subsequent milk production and income. During the dry period mammary glands undergo a number of changes that are necessary to stimulate maximal milk production during the subsequent lactation. The time period needed for resolution is the major factor determining the optimum length of the dry period. This is a very important topic. Yet, few studies have been completed and published to evaluate the problem. Heretofore, anecdotal and experimental data suggested that 7 to 10-wk dry periods were necessary to maximize maximal production. Thus, it is important to understand the changes that occur during the resolution process that leads to the necessity for a dry period, especially for a longer dry period.

Involution

After frequent periods milk removal from the mammary gland is discontinued the individual mammary glands undergo involution. Three types of involution have been described (Lawler and Lee, 1973). Gradual involution occurs during the declining phase of lactation after the peak milk yield has been reached. Involved involution describes regression of the lactation function with relative cessation of milk removal either natural or induced by the dairy producer. Finally, acute involution occurs at the end of the reproductive life of the animal.

Involved involution of mammary glands occurs after cessation of milking. Regression of mammary secretory tissue is accompanied dramatic changes in mammary composition during the transition from lactation to a non-lactating gland. As described above, it has been shown that dairy cows require a resolution period prior to the next lactation to achieve maximal milk production during that lactation (Cappola et al., 1974). Adequate proliferation and differentiation of mammary secretory epithelium during the resolution period are essential for optimal mammary function during the subsequent lactation and duration of the resolution period is related significantly to milk production (Allen and Hokeness, 1963). Smith and Tollerance (1962) suggested that there are three important stages during a typical dry period. The first stage is one of active involution which begins with cessation of milking and is completed within 21 to 28 d. This stage is characterized by regression of mammary tissue, duct involution with milk constituents, gradual changes in mammary secretory composition, and regression of mammary tissue. The second stage is that of steady state involution representing fully involution mammary gland. The final stage represents cellular formation and maturation

of lactation which begins about 14 d before parturition. Near parturition, mammary glands undergo significant changes characterized by intense growth, rapid differentiation of mammary epithelial cells, and synthesis and secretion of proteins: fat and carbohydrates leading to accumulation of colostrum. Thus, according to this view, a 45 to 60 d dry period would represent an active period of involution and the involution phase was completed, followed by redevelopment of mammary gland beginning 11 to 22 d prior to parturition (Bart and Moore, 1955; Makarewicz and Albers, 1989).

Much of the information on involution has been based on research conducted using laboratory animals. In rodents, continuous milk production during lactation is dependent upon a complex interplay of lactogenic hormones and the suckling stimulus exerted by the young. Involution can be initiated in the mouse mammary gland at any stage of lactation by removing the pups (Richards and Brown, 1971). Cessation of suckling causes accumulation of milk in alveoli and ducts, and this increases intramammary pressure that causes degeneration of mammary cells and subsequent disruption of alveolar and intralobular structures. In rat, involution is associated with a massive regression of the gland with cells followed by apoptosis of mammary epithelial cells and destruction of the gland. Involution appears reversible for about 30 to 35 d after it has been initiated (Richards and Brown, 1971).

After weaning, the decline in lactogenic hormones and milk cause leads to involution: a process that is widely characterized by three events: (i) downregulation of milk protein gene expression, (ii) loss of epithelial cells by apoptosis, and (iii) tissue remodeling and preparation of the gland for a new lactation. Each of these processes is likely to depend upon the activity of specific sets of transcription factors in the mammary

epithelium and stroma that ensure the timely and spatially-coordinated expression of critical gene products (Jelks et al., 2000).

In the mammary gland, secretory-epithelial cells are removed by apoptosis during involution. A number of transcription factors were found to control apoptosis. These include c-Fos, c-Jun (Cobelli et al., 1992), p53 (Yoshida (Rusch et al., 1990), E2F (Qian et al., 1994), Myc/Max (Kane et al., 1993) and STAT3 (Liu et al., 1996). STAT3 was reported to be activated during pregnancy and inactivation at the onset of involution. This activation was abrogated by removal of epithelial cells by apoptosis (Liu et al., 1996, Walker et al., 1995). STAT1 also is activated during later stages of involution when there is remodeling of the mammary gland (Liu et al., 1996). The timely breakdown of extra-cellular matrix is essential for remodeling (Haguen and Weaver, 1995). The cell loss coincides with matrix metalloproteinase (MMP) activation and basement membrane degradation *in vivo*.

Using *in vitro* culture, it was demonstrated that first passage epithelial cells isolated from pregnant mouse mammary gland die by apoptosis (Pallen et al., 1994). On the other hand, cell death was suppressed by basement membrane suggesting the requirement of basement membrane for cell survival (Pallen et al., 1994). In the same experiment, blocking of integrin with anti-beta 1 integrin antibody doubled the rate of apoptosis, whereas expression of beta-2 did not correlate with cell survival. However, increased levels of Bcl-2 were associated with apoptosis. Thus, basement membrane provides a survival stimulus for epithelial cells *in vivo* and loss of interaction between cells and this type of matrix may act as a critical point for cell-deletion during mammary gland involution (Pallen et al., 1994).

In fact, mouse and rabbit, macrophages and histiocytes/macrophages play a very important role in tissue degeneration during mammary involution (Dicks et al., 1987). Evacuation of basement and connective surrounding cytoplasmic separation (cytotactin) have been interpreted as evidence of endophagocytosis of alveolar cells. This effect was accompanied by detachment of epithelial cells from the basement membrane (Richards and Brown, 1971a), whereas infiltration of mononuclear leukocytes into involution tissue has been associated with histophagocytosis of degenerating cells and cellular debris (Richards and Brown, 1971a). Thus, involution of the rodent mammary gland is distinguished by the separation of epithelial cells from the basement membrane and entrance of macrophages to fill the gaps left by the detached epithelial cells (Dicks et al., 1987).

Ruminant mammary epithelial cells, on the other hand, apparently do not regress to the same extent as occurs in rodent mammary glands. They appear to maintain some cytochemical and secretory activity throughout the involuting period. This conclusion is based upon conditions of mammary involution in dairy cows that generally are considerably different from laboratory species (Dicks et al., 1987; Scollin and McLennan, 1988). Dairy cows still are producing large quantities of milk and most often they are pregnant at the time of milk cessation by natural involution or stoppage of milk removal (Oliver and Scollin, 1984). Even in the absence of pregnancy, mammary involution in dairy animals occurs at a slower rate than in rodents (Li et al., 1989). Alveolar structure is maintained for several weeks and lactation can be reinitiated after 4 wk or more of involution. Although apical cells in the mammary tissue of cows appear to be retained, what is unclear from these is that in rodents beginning about 2 weeks cessation of

milking or parturition with a peak at about 8 d, the maximum proportion of apoptotic epithelial cells appears to be less than in lactation, and apoptosis may be accompanied by an active increase in cell proliferation (Capuco and Adams, 1999). Adipose structure of cows is largely maintained and little or no loss of cells occurs with cessation of milking. On the other hand, there is increased apoptosis and cell proliferation in freshly dried off mammary glands, relative to that in lactating glands during the same stage of gestation. Thus, it appears that a remodeling process serves to prepare cell turnover prior to the next lactation (Capuco and Adams, 1999). In contrast to the view that suggests an active resolution process during dry period requiring 45 to 60 d in dairy cows (Smith and Telford, 1982), most recent studies (Capuco et al., 1997; Capuco and Adams, 1999; Li et al., 1999) imply that the dry period is important for replacement of damaged cells before the next lactation starts but not for extensive degradation of mammary gland structure and apoptosis. This suggests that 45 to 60 d dry period may not be the optimal time interval for maximum production of dairy cows and that the length of the dry period may be shortened without a negative effect on the cow's subsequent lactation performance.

Isabelle and Markman (1987) examined the morphologic changes in the mammary glands of 3 cows during mammary involution. Mammary tissue samples were obtained weekly beginning the day milking was discontinued through mastectomy. As involution progressed, a gradual reduction in synthesis and secretory activity of alveolar epithelium was noted under light and electron microscopic evaluation. During the first 3 wk of involution they observed sustained dense and extensive secretory epithelium with concomitant decreases in epithelium height and fully active secretory epithelium. There

was a decreased number of irregular anastomosed web duct systems and increases in the alveolar epithelium level. These changes were gradually reversed beginning 2 wk before parturition and by the time of weaning normal cell structure was typical of the lactating mammary gland.

Free fatty acid levels in milk increased more than 10-fold in cows during mammary involution (Thompson, 1989). Their appearance did not immediately follow the cessation of milking, but followed the increase in permeability of the mammary epithelium which paralleled changes in the electrolyte content of the milk. However, the concentration of free fatty acids did not remain high throughout the dry period but declined to low levels before the change in permeability was reversed at distant parturition. They concluded that the high level of free fatty acids in milk during mammary involution were likely a result of breakdown of triglycerides occurring in the gland and that maybe accelerated in some measure by the increase in permeability of the mammary epithelium (Thompson, 1989).

Lactating gland morphological studies in goat by Le et al. (1999) showed highly packed secondary ducts with columnar-shaped alveolar cells that released a large apical secretory vesicle. Secondary ducts were separated by small amounts of connective tissue. After drying off, the involutional gland showed a lactating morphology for 3 d with reduced number of alveolar cells and more intralobular connective tissue around the alveoli. However, less than 1% of alveolar epithelial cells underwent apoptosis as determined by both presence of TUNNEL-positive cells and DNA fragmentation in tissue extracts. The lumen of involutional alveoli contained residual secretions and polymeric triglycerides that occluded the parathyres. Morphology of the involutional gland changed 7

distal cells ventral aspect. Alveolar cells lost their columnar shape and the cytoplasm contained a large apical vacuole with a compressed ill-defined nucleus. Apoptotic bodies were observed and squamous cells (2-7% of alveolar cells)/fibres were absent. During the second week, the squamified cells were pronounced and formed a band around each alveolus. Body deficient cells were present among the structurally deficient alveolar cells which had retained cell membranes with an intensely stained pyknotic nucleus. Apoptotic cell rate was estimated to be 7%. Three weeks after the initiation of milking, fibrocollagenous tissue reappeared the ducts and alveolus. Apoptotic bodies were present in most ductal structures and not around the secondary pseudocysts with absence of body deficient cells. In this study (Li et al., 1997) apoptosis was not limited to the dry glands. Infrequent TUNNEL positive cells and low level of DNA fragmentation also were observed in milking glands suggesting apoptotic cells could have contributed for the net decrease in mammary cell numbers of the goat during lactating lactation.

Cover et al. (1997) concluded that, in contrast to rodents, no net loss of mammary cells occurred during the dry period in dairy cows. In their experiment, dry and lactating cows were introduced on days corresponding to different number of days into the dry period and mammary tissues were sampled for total DNA and RNA and morphological analyses. Neither pseudocyst weight nor DNA content differed in glands of lactating or dry cows. Seven days into the dry period, DNA content was identical in glands of dry and lactating cows and subsequently increased more rapidly in dry glands than in lactating glands. More squamified cells (30%) were labeled with [3 H]TdR in dry glands than in lactating glands (30%). Based upon morphology, 17% of squamified cells in lactating glands contained secondary vacuoles and lipid droplets and these were not

affected by day of parturition. However, if cows had been dry for 7 d, 33% of epithelial cells appeared to be secretory. At 33-d postpartum (post-dry for 33 d), none of the epithelial cells were secretory. Cells showing secretory activity increased to 75% and 95% after d 33 and d 7, respectively. They concluded that processes of proliferation and cell turnover increasingly increased the percentage of epithelial cells in dry mammary glands prior to parturition (Capuco et al., 1997).

Earlier studies suggested that along with the changes in composition of mammary secretions, mammary gland was fully reabsorbed during the steady state involution phase in cows (Smith and Todhunter, 1942). During this process, it was believed that total destruction of the gland occurred. However, as a result of more recent studies, it has been suggested that mammary involution is an inappropriate term to describe changes that occur in the bovine mammary gland during the dry period. Current evidence not support a net loss of mammary cells during the characteristic 60 d dry period in dairy cows (Capuco et al., 1997). Apparently, the bovine mammary gland does not degenerate to the extent that it does in rodents during the dry period. The structure of alveolar structures still can be seen 30 d following milk onset (Capuco et al., 1997; Holst et al., 1997). Earlier, it has been suggested that the dry period is necessary to allow replacement of damaged or senescent epithelial cells prior to the subsequent lactation (Capuco et al., 1997). Because mammary gland completed involution by 33-d dry (Capuco et al., 1997), it may not be necessary to have 60 d dry period in dairy cows. Thus, it is possible that the dry period could be shortened to 30 to 33 d without an effect on subsequent milk production. However, this conclusion implies that they have regained adequate BCS and replacement of body tissue reserves that will be needed during the subsequent lactation.

Plasma and Isolation

Throughout lactation, milk contains a number of proteins such as lactoglobulins, serum proteins, milk and plasma and plasmin (Auluck and Bailey, 1998). Plasma has been implicated as the dominant plasma factor against antibodies because most proteolytic activity found in milk is attributed by plasmin (Polzin et al., 1989). Plasmin is an extracellular serine protease that is formed by cleavage of a peptide bond in the single polypeptide chain of the inactive proenzyme plasminogen (Anderson et al., 1998). Plasmin generated in the extracellular space is isolated by cellular release of the single chain form of the plasminogen activator (PA) and their subsequent activation is responsible for degradation of fibrin (Thornes et al., 1984). Components of the plasmin system often are associated with various maculles but also are present in the serum and cross plasma of milk (Polzin et al., 1983). Plasmin appears to be responsible for fibrinolysis and rheumatolysis, as well as for biological processes involving breakdown of cellular matrix and basement membranes such as cell migration, angiogenesis, organ involution, tissue remodeling and destruction (Meyer et al., 1998).

The plasmin system is believed to have a role in the mammary gland during involution. Plasmin and its inactive precursor, plasminogen, are two of the several significant proteins in bovine milk (Lipari, 1977). Plasmin in bovine milk exists mostly in its inactive form. Plasminogen activation in milk converts plasminogen to plasmin (De Krom and Anderson, 1982). Stage of lactation affects plasmin with late lactation associated with higher concentrations of plasmin (Polzin et al., 1989). Potential mechanisms responsible for increased milk plasmin include an influx of plasminogen from blood (Polzin and Heng, 1985). In the richest mammary gland, PA converts

plasminogen to plasmin during late lactation and this is associated with the onset of involution (Chenais et al., 1979). Activities of plasminogen in bovine milk increases as lactation progresses and plasminogen activity increases further by d 3 after drying off (Polans et al., 1990). Elevated plasmin activity during involution of the bovine mammary gland is responsible for increased hydrolysis of casein and lactoferrin and protease activity other than elevated plasmin is assumed to play a major role in protein hydrolysis during involution (Adam and Hawley, 1988). It has been suggested that the increased plasmin activity seen during late lactation may be involved in subsequent mammary gland involution (Polans et al., 1990) and uterine-associated plasmin activity in the mammary gland (Auber et al., 1986).

Increased plasmin and PA in milk are considered during the declining phase of lactation. It has been suggested that mammary gland involution can be partially reversed by MMT administration via modulation of the plasmin-plasminogen system (Polans et al., 1990). Treatment with MMT correlates with conversion of plasminogen to plasmin and this prevents the increase of plasmin in milk (Polans, 1990). Balch (1984) studied the effect of MMT treatment on the plasminogen system in late lactation dairy cows. Even though plasmin level was not affected, there was a significant reduction in plasminogen activity in these MMT treated cows. Moreover, the plasmin:plasminogen ratio decreased in treated cows, suggesting a stimulation of conversion of plasminogen to plasmin in these animals. It has been suggested that MMT may preserve the integrity of tight junctions in late lactation because the plasminogen in milk is derived usually from the blood (Balch, 1984).

Materials and Methods

The second phase of the study reported in Chapter 3 was designed to evaluate concentrations of hormones (IGT and IGF1, growth factor (IGF-1) and metabolites (Lactate and NEFA) in plasma of dairy cows during the period beginning 24-d before parturition and extending through 24-d postpartum. Blood samples were collected from 10 Holstein cows from the research herd at the UCD. Description of experimental animals, management, feeding program, drying off times and IGT exposure were described in detail in chapter 3. Plasma harvested from blood samples collected throughout the experiment were frozen at -20°C until analyzed.

Milk Sampling

Milk samples were collected weekly during three consecutive milkings (8h 30, 12 00, and 16 30 h) on same day of the week for analysis of milk constituents during the first 18 wk of lactation. Samples (50 mL) were analyzed for fat, protein and IGT contents at Southern Milk Laboratory Inc (Bellevue, PI). Milk yield was recorded at each daily milking from 1-d after parturition through 24-d postpartum.

Plasma Collection, Handling and Storage

Blood samples were collected from the tail vein of all cows three times weekly before the a.m. feeding or milking (07 30-10 00 h). Cows were tied from the tail vein in the free stall barn after elevating the tail without any other restraint. For blood collection, Vacutainer® blood needles (2.44 cm, 20 gauge) and tubes containing gelatin heparin were used (3.8 x 100 mm blood collection tubes, Becton-Dickinson, Franklin, NJ). Blood samples were placed on ice immediately after collection and processed within 2 h. The order in which cows were sampled on a given day was random and differed from

bleeding to bleeding. After sampling, cows were milked and then returned to the free-stall barn.

All samples of blood were centrifuged at 3000 RPM at 4°C for 30 min in the RC-30 refrigerated-centrifuge (4-place swinging-bucket, 5-600A rotor, Sorvall Instruments, Wilmington, DE) in separate places. Plasma from each sample was aliquoted into 1.5-ml Eppendorf (Fisher Scientific) polypropylene tubes, capped, and frozen at -20°C until analyzed. The plasma samples were used for analysis of ET, DMS, KGF-1, plasmin and HETFA.

Second Antibody Preparation

Second antibody for use in radioimmunoassays was prepared in four Florida. Mature sheep managed at the DRI/ University of Georgia goats (20-25 kg, Sigma Chemical Co., St. Louis, MO # R-9134) and 30-40 mg of rabbit gamma globulin (Sigma Chemical Co., St. Louis, MO # R-9134) were weighed into separate 25-ml Erlenmeyer flasks, then 3-ml 5 mL of distilled water were added to each. After proteins had dissolved an equal amount of (Pierce's) complete adjuvants (1st injection only) or incomplete adjuvant (2nd and greater injections) was added to each flask and it was injected in a quadrilateral. For about 1 hr at high speed until the mixture had the consistency of whipped cream. Sheep were injected over the shoulder or back-left and right sides with about 3 mL of a mixture of the two proteins. After 14-d, each was bled from the jugular vein to obtain about 400 mL blood. This was done using a 16-gauge butterfly needle inserted into jugular vein; a filter syringe was attached to the tubing attached to the needle to withdraw the blood. Blood was transferred to 40 mL serum-separation tubes and placed on ice as soon as it was collected. Tubes then were refrigerated for 24 hr at 4°C so that blood

to clot. The clotted blood was centrifuged at 3000 RPM for 10 min in the RC-1B refrigerated centrifuge (8-place swinging basket, H-500A, rotor (Sorvall Instruments, Wilmington, DE) to obtain serum. Serum was frozen until used as source for second antibody in appropriate dilutions. Sheep were bled again at 4 wk or greater intervals. They were injected with mixture of gamma globulins at about 4-6 mo intervals.

Radioimmunoassay

Double antibody radioimmunoassay procedures were used to determine concentrations of DMS, ST, and IGF I in plasma. All samples from individual cows were assayed in duplicate in a single assay.

Isolation and Protein Separation

Isolation

Bovine DMS (400-500 μ g, Sigma Immunochemicals, St. Louis, MO, DMS 8413) was weighed and mixed with an equal quantity of 3 mM DCl. Then, an equal amount of 0.01 M borate buffer (pH 8-9) was added to the solution to give a final concentration of 0.5 μ g DMS/ μ L buffer. 10 μ L of this solution were frozen in microcentrifuge vials (Fisher Scientific, 1/8 in. flat top).

The column used to separate the solubilized protein from this solution was prepared by cutting off the mouthpiece of a disposable 10-mL glass pipette. A small glass wool plug or glass bead was placed into the column before adding the Sephadex G-10 packing (Sigma Chemical, dispersed in 0.05 M phosphate buffer, pH 7.3) until the Sephadex filled the column. Subsequently, the column was washed with 2 mL 0.5 M phosphate buffer which contained 0.1% BSA followed by 10 mL of 0.05 M phosphate buffer. Phosphate buffer was returned at the top of the Sephadex bed until used for separation.

Immediately prior to the reactions, 3 mg chloroform- T and 3 mg sodium pyrophosphate were weighed into individual tubes and just prior to use each was dissolved in 1 ml. of 0.5 M phosphate buffer. Then, 1 mCi (3.0 μ L) ^{32}P was transferred to a reaction tube (12 x 75 mm) containing 10 μ L BPS and then 10 μ L of 0.5 M phosphate buffer was added. After mixing, 10 μ L of chloroform- T were added to the reaction tube, contents were mixed with finger tapping for 20 sec after which 10 μ L of sodium molybdate were added in the same way for reaction. Separately, the solution containing BPS- ^{32}P was transferred to the top of the Sephadex column; the reaction tube was mixed with 20 μ L of 0.5 M phosphate buffer and then also was transferred to the top of the column and allowed to flow into the Sephadex bed.

Reaction tubes (12 x 100 mm), numbered 1-50, that contained 100 μ L of 0.5 M Tris-HCl buffer, were used to collect 20-drop fractions using a fraction collector (model FC-45 E, from Beckman-Coulter, Culver Medical, Middlesex, NY). The fractions collected were mixed by finger tapping, then 10 μ L of each eluted fraction were transferred to a second set of tubes (12 x 75 mm) to identify radioactive peaks by running each individually using a Tracer Analytic Gamma Counter (Model-4000, Gamma Trac 1191, G.D. Scatchard Co., Des Plaines, IL). Tubes from the first large peak corresponding to ^{32}P bound BPS were saved and stored at 4 C until used in the radioassays.

Radioassay

One well containing 1 μ g of BPS-4 (0.5 μ g/ μ L) was allowed to dry at room temperature. The microspot of a 10 μ L disposable glass pipette was cut off with a glass tubing cutter, and then a small glass microjet or glass bead was placed into the bottom

of the glass pipette. Sephadex G 30 (Pharm Chemical, St. Louis, MO) dispersed in 0.01 M phosphate buffer was transferred into the pipette until the Sephadex was between the 0 and 1 mark. Subsequently, the column was rinsed with 2 mL of 0.01 M phosphate buffer containing 0.5% BSA, followed by 10 mL of 0.01 M phosphate buffer. Phosphate buffer was retained at the top of the Sephadex bed until the column was used for separation.

Polystyrene monomethylololigo tubes (Fisher Scientific, 10 cm), that had been washed with glass ionite acid, rinsed with deionized water, and allowed to dry (0.5 mg of iodogen (Pierce Chemical Co., Rockford, IL) was weighed and dissolved in chloroform to a final concentration of 100 µg/mL) then 20 µL of iodogen solution were transferred directly onto the bottom of the acid washed monomethyloligo tubes followed by 40 µL of eluent/diluent. The chloroform then was dried under a stream of air to leave the iodogen coated on the inside of the tube. For the columns, 10 µL of 1×10^{-6} M IGF-1 followed by 20 µL of 0.01 M phosphate buffer were added to the polystyrene monomethyloligo tube and rinsed with 10 µL of 1×10^{-6} M IGF-1 and allowed to react for 5 min. Subsequently, IGF-1¹²⁵ solution was transferred to the top of the column. The column tube was rinsed with 20 µL of 0.01 M phosphate buffer and it also was transferred to the top of the column and allowed to flow into the Sephadex bed.

Barbiturate tubes (1.5 x 100 cm) numbered 1-40, containing 500 µL of 0.01 M phosphate buffer were used to collect 10-drop fractions using a fraction collector (model FC 40-K micro-fraction collector, Gilson Medical, Middleton, WI). The fractions were assayed by Rager tapping, then 10 µL of each eluted fraction were transferred to a second set of tubes (1.5 x 15 cm) to identify elution peaks by measuring radioactivity using a Tracor analyte gamma counter (Wallac-Compac, Gemini Trac 1101, G. D. Searle and Co., Des

Plasma, IL-1. Taken from the first large peak corresponding to P^{32} -bound IL-1 was pooled and stored at 4°C until used for radioimmunoassay.

Separations

Bovine ST (100–200 μg , USDA, Reproductive Lab, APP 3088) was weighed and diluted with an equal quantity of 0.04 M NaHCO_3 . Then an equal amount of 0.04 M phosphate buffer was added to the solution to yield a final concentration of 0.5 μg ST/ μL solution. 10 μL of the homogen solution were frozen in microcentrifuge vials (Fisher Scientific, 1.5 mL, flat top) for use as standards.

The column used to separate the isolated ST from free iodine was prepared by cutting a filter manglepiece of a 10 mL disposable glass pipette. A small glass wool plug or glass bead was placed into the bottom of the glass pipette. Sephadex C-75 (Sigma Chemical) dispersed in 0.04 M phosphate buffer was transferred into the pipette until the sephadex was between the 0 and 1 mark on barrel of the pipette. Subsequently, the column was rinsed with 2 mL of 0.04 M phosphate buffer containing 0.1% BSA followed by 10 mL of 0.04 M PQ_4 buffer. Phosphate buffer was retained at the top of the Sephadex column and used column was used for ST separations.

Thirty ng chloroform-T and 5 ng sodium metabisulfite were weighed into individual tubes and each was dissolved in 1 mL of 0.04 M phosphate buffer immediately prior to the reactions. One mL (10 μL) P^{32} was transferred into a conical plastic centrifuge tube containing 10 μL of ST solution and then 10 μL of 0.04 phosphate buffer was added. After adding 10 μL of chloroform-T was added to the reaction tube and contents mixed with finger tapping for 30 sec after which 10 μL of sodium metabisulfite were added to stop the reaction. Subsequently ST- P^{32} solution was transferred to the top

of column, the reaction vial was rinsed with 50 μ L of 0.05M phosphate buffer and this also was transferred to the top of the column, and transferred substances were allowed to flow into the Sephadex bed. Borosilicate tubes (3 x 100 mm) numbered 1-45, were filled with 500 μ L of 0.05 M phosphate HSA buffer and then 20 drops fractions were collected in the tubes using the fraction collector (model FC-40 E, from Pharmacia, Cetus Medical, Maitland, NY). The fractions were mixed by finger-tapping, then 50 μ L of each eluted fraction were transferred to a second set of tubes (12 x 75 mm) to identify elution peaks by scoring radioactivity using a Thermo Analytic Gamma-Counter (PacStar, Chicago, Gamma Inc. 119), G.D. Scarle and Co., Des Plaines, IL). Tubes from the first large peak corresponding to 125 I-ST were saved for radioimmunoassays and stored at 4°C until used in the assays.

Assays

Radioimmunoassay

A double antibody radioimmunoassay procedure, as described by Scatchard and Monod (1955), and modified by Mahesh et al. (1974) was used for assay of PGE in plasma. Highly purified PGE (Upjohn Laboratories, St. Louis, MO 1117-4424) was weighed ($> 100 \mu$ g), then dissolved in 30 ml of HCl (pH 2.5). The PGE was (10 μ g/30 μ L) aliquotted into 1 mL, storage vials and frozen until used. Stock PGE was diluted in borate-HSA assay buffer (pH 5.0) to obtain 0.01%, 0.05%, 0.1%, 0.2%, 0.5%, 1.0%, 2.0%, 4.0%, 8.0%, 16.0%, 32.0%, and 64.0 μ g PGE/ml were prepared and frozen in 1 mL aliquots. For preparation of free antibody, Gamma pig anti-human PGE (Upjohn Chemical CO., St. Louis, MO) was dissolved in borate-HSA buffer

(1:20,000). Second antibody (anti-guinea pig anti-sheep serum) was diluted 1:4 in bovine BSA buffer (1.16 g BSA in 100-ml bovine buffer).

Arrangement of assay tubes: see Table 4-1. Plasma samples (150 μ l) were assayed in duplicate along with 150 μ l bovine buffer (0.1% BSA) on 12 x 75 mm borosilicate tubes and 100 μ l first antibody (except for tubes for total count, NGB-B, and NGB-F) were added. Then, 11% (w/v) 100 μ l red-stained DCS (+ 25,000-CPM) were pipetted into all tubes. After a 30-min incubation at 4°C (starting from addition of first antibody) 100 μ l diluted sheep anti-guinea pig second antibody (SA-CP, 1:4 dilution) and 100 μ l normal guinea pig serum (1:100 dilution) were added to all tubes except for total count tubes. Tube contents were mixed then allowed to stand for 10 min.

Table 4-1. Arrangement of Assay Tubes for DCS

Tubes ^a	Samples	Buffer ^{b,c}	1 st Antibody	1 st DCS	Plasma
TCT	---	---	---	100	---
NGB-B	---	400	---	100	---
NGB-F	---	250	---	100	150
ZERO	---	300	100	100	---
STANDARD	100	200	100	100	---
SAMPLES	150	150	100	100	---

^aTCT=total activity count tube, NGB-B=non-specific binding for bovine, NGB-F=non-specific binding for plasma, ZERO=reference, no DCS added. ^bbovine 0.1% BSA buffer. Volume is in μ l. ^c---

Following the 10 min incubation 750 μ l of 10% polyethylene glycol (PEG,

Boehr Mannheim GmbH (St. Louis, MO) in bovine buffer were added to all tubes except for the total count tubes and tubes were vortexed for 1 min. Tubes then were centrifuged

assay tubes, and mixed (Table 4-2). Then, 1.8 ml. of 4M PEG(Sigma Inorganic Chemicals, St. Louis, MO) in 0.04 M phosphate buffer was added to all tubes, except total count tubes, and vortexed for 1 min, mixtures were incubated for 10 min, then all tubes were centrifuged at 3000 RPM at 4°C for 10 min(BC-38 refrigerated centrifuge with 6 place swinging bucket, B-EHBA rotor, Sorvall Instruments) decanted, and allowed to dry in inverted position. Bound radioactivity in the dry tubes was measured with a Packard 3 min gamma counter (model B-5800). Final results were calculated using the spleen radioimmunoassay data processing procedure as a coded assay to correct for any differences that may exist in NGB-B and NGB-F.

Table 4-2. Arrangement of Assay Tubes for ST

Tubes ^a	Samples	Buffer ^{b,c}	1 st Antibody	1 st ST	Plasma
TCT	---	---	---	100	---
NGB-B	---	400	---	100	---
NGB-F	---	200	---	100	100
ZERO	---	200	100	100	---
STANDARDS	100	200	100	100	---
SAMPLES	100	200	100	100	---

^aTCT-total activity-count tubes, NGB-B-non-specific binding for buffer, NGB-F-non-specific binding for plasma, ZERO-reference, no ST added. ^bPHOSPHATE BUFFER Volume are in µl.

RESULTS

A double antibody radioimmunoassay as described by Albers et al. (1980) and modified for sample extraction by method of Wright et al. (1980) and Daugherty et al. (1980), was used for EGF-R determination in plasma samples.

Extraction of IGF-I from human plasma. The method of Bougati et al. (1989) was used for the extraction of IGF-I from six healthy persons in plasma samples. An extraction mixture of ethanol, acetone, and acetic acid (EAA 85/15) (v/v) was used for extraction. Exactly 100 μ L plasma (300 μ L distilled water for IGF-PI) and 400 μ L of extraction mixture were pipetted into 12 x 75 polystyrene tubes. Tubes contents were mixed for 15 sec on a vortex and then were allowed to stand for 30 min at room temperature. Tubes then were centrifuged at 3000 RPM for 30 min at 4°C (SC 10B refrigerated centrifuge with 8 place swinging bucket, H-6000A, mixer (Sorvall Instruments). Then 150 μ L of the supernatant were transferred to polystyrene tubes (2 x 75) and 400 μ L of 0.150 M acetic acid and 150 μ L of the assay buffer were added to make the final dilution 1:14.

Assay. Highly purified human insulin like growth factor-I (IGF-I) supplied by Lysate Technologies (Lake Placid, NY) (IGF 50:100) was dissolved (10 μ g) in 100 μ L of 0.1 M acetic acid to give stock 0 (100 ng/mL). This was aliquoted into microcentrifuge tubes (10 μ L/tube) and frozen. To make stock 1, 10 μ L of stock 0 were added to 490 μ L of assay buffer. Stock 2 was made by adding 10 μ L of stock 1 to 990 μ L of assay buffer to give a final concentration of 20 pg IGF-I/mL. Standards were prepared from stock 2 to contain 30, 100, 300, 1000, 6000, 8000, 10000, 12000, 15000 and 20000 pg IGF-Peptide. The first antibody, rabbit anti human IGF-I (Lot #AHP440390), was dissolved (1:140000) in assay buffer (200 mg potassium, 4.4 g sodium monobasic phosphate, 10 mL of 7% sodium azide, 5.73 g EDTA and 2.5 g BSA in 1 L). The IAA was diluted 1:3 in EDTA. Twenty μ L of plasma extract were mixed with 100 μ L assay buffer. Then 100 μ L of diluted IGF-I were added to all tubes, and 100 μ L of first antibody were added to

references, standards, and samples but not the MSD tubes. All tubes were incubated for 24 h at 4 °C. After incubation, 20 µL second antibody diluted 1:1 in assay buffer and 10 µL, diluted whole serum (1:50) were added to all tubes except the total assay tubes. Samples were allowed to react 30 min, and then 1 mL of 1% PEG (polyethylene glycol, St. Louis, MO) in assay buffer was added. Tubes were vortexed and allowed to stand 15 min, and then they were centrifuged at 3000 RPM for 30 min at 4 °C (RC-50 refrigerated centrifuge H-505A rotor (Sorvall Instruments)). Finally, supernatant in tubes was discarded and tubes were inverted on absorbent paper to dry. Finally, bound radioactivity in the dry tubes was measured using a Packard® scintillation counter (model B-5000). Final results were calculated using the spline radioactivity-count-rate processing procedure for a coded assay to correct for any differences in NDEs for plasma extract and buffer.

Table 4.1 Assay Format of Assay Tubes for KGF-1

Tubes ^a	Samples	Buffer ^{b,c}	1 st Antibody	1 st PEG	Plasma Extract
TCT	—	—	—	100	—
MSB-B	—	100	—	100	—
MSB-P	—	100	—	100	20
ZERO	—	100	100	100	—
STANDARD	100	100	100	100	—
SAMPLES	20	100	100	100	—

^aTCT=total activity assay tube, MSB-B=non-specific binding for buffer, MSB-P=non-specific binding for plasma extract, ZERO=reference, no KGF-1 added

^{b,c}“plasma”=MSB/buffer. Volumes are in µL.

Determination of Glucose in Plasma Samples

Sigma procedure No. 166 (Sigma Diagnostics, St. Louis, MO) was used for the quantitative enzymatic determination of glucose in deproteinized plasma samples as described by Basso and Bertolotto (1969).

Enzyme solution for the assay was prepared by adding one capsule of PGO enzymes, which contained 500 units of glucose oxidase (coding no. 518-4) to 100 ml. deionized water in an amber bottle. Color reagent solution was prepared by reconstituting one vial of α -D-glucose 1-Dehydrogenase (coding no. 518-50) with 20 ml. deionized water. Then, 100 ml. of enzyme solution and 1.8 ml. of color reagent solution were mixed by mild shaking.

Glucose standards (0, 25, 50, 75, and 100 mg of glucose/dL) were prepared by diluting the glucose standard solution provided (100 mg/dL) with deionized water to achieve the desired standard concentrations (Table 4-1). Along with standards, 100 μ L of unknown plasma samples were pipetted into borosilicate tubes (Fisher Scientific, Pittsburgh, PA). Then, 900 μ L of distilled water were added to bring volume to 1 mL. Plasma was deproteinized by adding 500 μ L barium hydroxide solution (0.3N) and 300 μ L zinc sulfate solution (5%) into all tubes, including standards. All tubes were vortexed for 30 sec and centrifuged at 3000 RPM for 30 min at 1-4 °C (BC-5B refrigerated centrifuge with H 600A rotor, Sorvall Instruments).

Ninety-six-well flat-bottom polypropylene micro-plates (8 20 mL capacity, Corning Inc., Herten, NJ) were used to complete assay. Standards and samples were assayed in triplicate and duplicates, respectively. Twenty microliters of supernatant (standards and plasma) were added to wells followed by 100 μ L of combined oxygen-

color reagent solution and plasma were incubated for 30 min at 37 °C at a constant temperature with (model 220-40, American Scientific Products). After incubation, the absorbance was read in an Automated Microplate Reader (Model EL 505, Bio-tek Instruments, INC., Laboratory Devices, Wisconsin VT) using Hach as reference at 490 nm wavelength. Linear regression of absorbance and glucose concentration was used to determine the concentration of glucose in plasma samples.

Table 4-4. Standards for Glucose determination

Standard (mg/dl)	Glucose Stock Solution (l. (mM))	Diluent (water)	Final Volume
0	0 μ L	400 μ L	400 μ L
25	100 μ L	300 μ L	400 μ L
50	200 μ L	200 μ L	400 μ L
75	300 μ L	100 μ L	400 μ L
100	400 μ L	0 μ L	400 μ L

Determination of NEFA in Plasma Samples

An enzymatic colorimetric method (NEFA-C, Wako Pure Chemical Industries, Osaka, Japan) was used for the quantitative determination of NEFA in plasma as described by Johnson and Peters (1992).

Color reagent A solution was prepared by adding 10 ml. of solution solution A and 13.7 ml. 50 mM phosphate buffer (4.4 g Sodium phosphate monobasic, 14.2 g Sodium phosphate dibasic in 500 ml. distilled water, pH 6.7) to one ml of dry color reagent A. Color reagent solution B was prepared by adding 20 ml. of diluent for color

reagent B and 33.3 μ L of 20 mM phosphate buffer to one mL of dry-ice reagent B. Vials containing reagents A and B were mixed gently and stored at 3 °C for up to 2 wk.

Specific concentrations of standards (0, 100, 400, 600, 800 and 1000 μ Eq NREs/L) were prepared by diluting the NRE's standard solution provided (3000 μ Eq/L) with 8.9% saline solution (3 g of NaCl in 1000 mL deionized water) to achieve the desired standard concentrations (Table 4-5). Ninety six well flat bottom polystyrene micro-plates (5-50 mL capacity, Corning Inc., New York, NY) were used. Standards and samples were assayed in triplicate and duplicate, respectively. Then, 2.5 μ L of supernatant (standards and plasma) were added to wells, followed by 50 μ L of Wako Reagent A and then were incubated for 30 min at 37 °C in a constant temperature oven (Cmodel 28-41, American Scientific Products). After incubation, 100 μ L of Wako

Table 4-5 Standards for NRE's determination.

Standards (μ Eq/L)	Dist. Stock Solution (3.0xM)	8.9% Saline
0	0 μ L	500 μ L
100	100 μ L	400 μ L
400	200 μ L	300 μ L
600	300 μ L	200 μ L
800	400 μ L	100 μ L
1000	500 μ L	0 μ L

Reagent B were added to the wells and placed in the oven for an additional 30 min at 37 °C. Microplate then was allowed to sit for 5 min on the bench at room temperature after which absorbance was read in an Automated Microplate Reader (Model EL 308, Bio-tek Instruments, INC., Laboratory Devices, Winooski, VT) using Mopha as reference at 550

the wavelength. Linear regression of absorbance and NEFA concentration was used to determine the concentrations of NEFA in plasma samples.

Statistical analysis

Data collected during the experiment were analyzed in two sections. The first section included data collected during the 21-d prepartum period. The second section included data collected during the 28-d postpartum period and during 150 d for MT. Data were analyzed using Proc GLM procedure to a nested design by least square analysis of variance procedures of SAS (1991). Additionally, Mixed model was used to compare specific least square means (Lalor et al., 2005). Statistical analyses were performed for BW and BCS, milk and 3.5 % FCM yields and concentrations of ST, NEFA, GLU, glucose and NEFA in plasma. Two periods considered for data analysis were the prepartum period (11 to -1 d), overall prepartum period (1 to 28 d) and 0-150 d postpartum period for MT. Models included the main effect of MT treatment (MT), effect of dry period length (DPN), effect of prepartum diet (DPT), season (SEA, 1=cows with dry periods during hot months (September, October, March, April, and May), 0=cows with dry periods during cool months (November, December, January and February)), interactions among the treatments and SEA, $\text{sex}(\text{MT} \times \text{DPN} \times \text{DPT} \times \text{SEA})$, and weeks or days in the highest order significant for overall prepartum and postpartum periods.

Regression analysis was performed to the highest order significant up to cubic order to describe the trends in measures during prepartum period and for MT during the overall prepartum period. Tests for heterogeneity of regression was performed to

determine whether there was evidence that regression differed with sex/period (Wilcoxon et al. 1999).

Specific models are described in the Results section. Significance was declared at $P=0.05$, except where noted.

Results

Objectives of this study were to evaluate changes in plasma concentrations of ST, INS, IGF-1, glucose and NEFA during the period of blood sampling from 23 d before calving through +28 d postpartum, and MT through 176 (20d). Plasma concentrations of ST, INS, IGF-1, glucose and NEFA and MT were analyzed for treatment effects [Sensitized treatment (control and expected dry period treatments) (28 d dry, 56 d dry + ICP or 80 d dry) prepartum and treatment (treated and untreated dry, and season effects)] Data were obtained from 15 Holstein cows for MT and 30 of them cows for blood analyses (treated were not bled) as described in Chapter 3.

Economics, Growth Factors and Metabolites

Prepartum period

Last square analysis of variance for all blood/plasma measures are in Tables 4-6 and 4-7. No differences between/ST treatment groups were detected for mean concentrations of glucose and NEFA during the overall prepartum period from d (2) to d (1, but differences were detected for the other measures. Significant effects of/ST treatment were detected for ST ($P=0.0015$), IGF-1 ($P=0.0001$) and INS ($P=0.012$) during prepartum period (Table 4-6).

Last square means and SE for all dependent blood/plasma variables during the overall prepartum period are in Table 4-8. Mean concentrations of ST during the overall

proportion pooled) were greater for MHT treated cows (29.0 ± 3.31 ng/mL) and increased concentrations were maintained throughout the proportion period (Figure 4-3).

Plasma concentrations of KGF-1 during the proportion period (from d -20 to d 0) were also in 4-8. Mean concentrations of KGF-1 during the last 5 wk proportion differed due to treatment ($P<0.01$). Cows in MHT treated group had greater mean plasma concentrations of KGF-1 than cows in untreated group (311.7 vs 233.2 ng/mL, 33.3%). Overall, plasma concentrations of KGF-1 decreased progressively from d -20 to parturition in both groups (Figure 4-3), mean concentrations for the treatments were 360.7 ng/mL (untreated) and 344.2 ng/mL (MHT), which corresponded to decreases of 36.9 and 25.9%, from d -11, respectively.

Mean concentrations of DMS also were significantly greater (24.7%) for MHT treated cows during the overall proportion period (d -13 vs 1-60 ng/mL, respectively, Table 4-4). Concentrations of DMS declined in both groups as they approached calving but concentrations remained low for cows in untreated compared to MHT treated throughout the proportion period. However, the decline appeared sharper beginning d -3 for cows in the untreated group because concentrations declined from greater to consistently the same during the week before calving (Figure 4-3).

Although overall proportion mean concentrations of glucose did not differ due to MHT treatment (Table 4-5), plasma concentrations of glucose increased significantly at d -14 in MHT treated cows and tended to stay higher through calving (Figure 4-4). Increase in mean plasma concentrations of glucose for the MHT treated group at d -1 was about 6.4% greater than d -13 and this was significant ($P<0.05$).

Mean concentrations of NEFA are in Table 4-8. Considering the overall preparation period (from d -21 to -3), mean concentrations of NEFA in plasma did not differ between the two lact treatment groups (263.0 vs.275.5 $\mu\text{g/L}$, respectively). Even though mean plasma concentrations of NEFA were steady during the preparation period, a significant increase was observed beginning d -3 preparation within groups and mean concentrations were greater toward calving for both groups (Figure 4-5).

No differences in mean concentrations of ST and MUFAs were detected among dry period groups during the overall preparation period. However, treatment differences were detected for IGF-1 ($P=0.0057$), DMS ($P=0.0044$) and glucose ($P=0.0448$).

Plasma concentrations of ST for the three dry period treatments did not differ during preparation period. Cows in 60-d dry period had the lowest mean concentration of ST (3.81 ng/mL), whereas cows in 30-d dry and 30 d dry + ECP groups had greater concentrations of ST but they did not differ significantly during the overall preparation period (7.17 ng/mL vs.7.48 ng/mL). Although cows in 30 d dry + ECP group had numerically greater mean concentrations of ST at d -21 and d -18, they did not differ significantly. Moreover, no differences were detected among the three treatment groups for any days of the preparation period (Figure 4-1). On the other hand, preparation mean concentrations of IGF-1 were greatest (121%) for cows in 30 d dry ECP group (309.8 ng/mL) whereas cows in 60 d dry and 30-d dry groups had lower mean concentrations (289.7 and 284.5 ng/mL, respectively Table 4-9). Cows in 30 d dry + ECP group had higher plasma concentrations of IGF-1 on d -21, but plasma concentrations of IGF-1 decreased progressively for all three treatment groups as cows approached parturition.

Table 4-4. Least Squares Means and 95% Confidence Intervals for Growth Percent and Mortality in Pigs of Various Crosses (Adjusted for sex) against early MT Treatment (Dependent) (4-41 to 4-44)

Blood Viscosity	Growth Percent (4-41 to 4-43)				Mortality (4-42 to 4-44)			
	MT Treatment ^a		S.E.	D.F.	MT Treatment ^a		S.E.	D.F.
	1	2			1	2		
GT (mg/dl.)	1.13 ^b	0.48	0.19	0	0.02	0.14	0.10	0
BSA (mg/dl.)	0.00 ^b	0.01	1.05	0	0.02	0.03	0.03	0
SGT (mg/dl.)	155.0 ^b	1.4	11.0	0	0.4	0.0	0.0	0
Glucose (mg/dl.)	49.1	1.0	7.4	0	1.0	0.1	0.1	0
MT (mg/dl.)	240.0	14.1	27.5	0	17.1	0.4	0.4	0

^a Treatment 1=No MT Treatment (0-25.2 mg MT/kg, 0-10 mg/kg); Treatment 2=25.2 mg MT/kg, 10-10 mg/kg.

^b 0-25.2 mg/kg MT Treatment only appropriate within the Population in Population treatment.

Overall, on d. 1, concentrations were about 40% less for all cases relative to concentrations on d. 25 (Figure 4-2).

Least squares means and SE of INS for cows in the three dry period treatment groups are in Table 4-5. Mean concentrations of INS during the overall preparation period were greatest for cows in 30 d dry that had been exposed with ECP (1.05 ng/mL). Plasma concentrations of INS were similar for cows in 30 d dry group (0.93 ng/mL) and 60 d dry group (0.84 ng/mL). Overall, concentrations of INS tended to decrease slightly from d. 25 to d. 1 in both 30-d dry period treatment groups (Figure 4-3). Mean concentrations of glucose in plasma during the same time period tended to parallel that of INS and concentrations also were greatest for cows in 30-d dry + ECP group (13.3 mg/dL) and least for cows in the 30 d dry (10.1 mg/dL) and 60 d dry groups (10.5 mg/dL, Figure 4-4). On the other hand, plasma concentrations of NEFA declined during the dry period treatment groups during the preparation period. All treatment groups had nearly plasma concentrations of NEFA during the preparation period until d. -8. Beginning at d. -8, there was a significant increase in concentrations of NEFA was observed within each of the three groups, with concentrations were greatest and similar around calving for all three treatment groups, but there was about a 3-fold increase around calving (Figure 4-5).

No significant effects of preparation diet was detected for ST, KGL, or However, plasma concentrations of NEFA were significantly higher (24 %, Table 4-5B) for cows on control group ($P < 0.005$) especially around calving.

Mean concentrations of KT during overall preparation period did not differ due to diet (control or mixture) fed preparation (Table 4-6). Cows fed the mixture diet had slightly, but not significantly, greater mean concentrations of KT than those fed control

Table 4.4: Least Squares Means and SE of Means for Growth Period and Metabolic Concentrations in Tissues of Broiler Chicks with 20 to 40 d Day Periods (A to D) (n=10)

Blood Measures	Periods (A to D)			
	Day Period Treatment ^a			
	I	II	III	IV
BT (mg/dL)	7.17 ± 0.04	1.44 ± 0.03	1.14 ± 0	0.90
BUN (mg/dL)	0.09 ¹ ± 0.04	1.09 ² ± 0.07	0.00 ³ ± 0.07	0.07
BCP (mg/dL)	200.3 ¹ ± 0.7	100.0 ¹ ± 10.1	200.1 ¹ ± 10.1	10.1
Cholesterol (mg/dL)	80.3 ¹ ± 1.1	50.3 ¹ ± 1.1	40.3 ¹ ± 1.1	1.1
ALP (U/L)	200.3 ± 1.1	200.1 ± 10.1	211.3 ± 1	10.1
	Periods (E to H)			
	Day Period Treatment ^a			
	I	II	III	IV
BT (mg/dL)	1.00 ± 0.03	0.41 ± 0.03	0.10 ± 0.09	0.09
BUN (mg/dL)	0.00 ± 0.03	0.03 ± 0.03	0.00 ± 0.00	0.00
BCP (mg/dL)	101.1 ± 1.1	100.7 ± 9.1	100.3 ± 9.1	9.1
Cholesterol (mg/dL)	0.00 ± 0.0	0.00 ± 1.0	0.00 ± 1.0	1.0
ALP (U/L)	100.1 ± 0.7	100.1 ± 0.0	100.0 ± 1	10.1

Treatments for 20 d day period, Treatment III 20 d day period + 40 d day period for 60, Treatment III 40 d day period for 60, Treatment III 20 d day period for 60, Treatment III 40 d day period for 60

Table 1.10 Total Squares Means and SE of Concentrations of Humulus, Growth Factors, and Metabolites in Plasma of Rabbits Given Full Nutrition Administered in Control (Full Diet), Fasting (F 0 to 4 24),

Blood Measures	Fasting (F 0 to 4 24)			Fasting (F 0 to 4 24)		
	Fasting (F 0 to 4 24)			Fasting (F 0 to 4 24)		
	1	2	3	1	2	3
SE (mg/dL)	7.28	a	0.10	8.43	a	0.49
SE (mg/dL)	1.88	a	0.26	8.31	a	0.86
ACE (mg/dL)	204.8	a	8.8	275.1	a	1.9
Glucose (mg/dL)	78.7	a	1.9	76.2	a	1.4
ACE (mg/dL)	204.2	a	0.3	203.4	a	11.6

†Fasting (Fasting during Day, Fasting 24-Hour Fasting Day).

‡Fasting (Fasting during Day, Fasting 24-Hour Fasting Day).

§Fasting (Fasting during Day, Fasting 24-Hour Fasting Day).

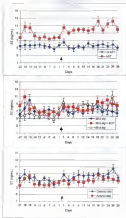


Figure 4-3 Least squares mean concentrations of IT in plasma during the transition period (21 d through 28 d). Jersey and Friesian cows.

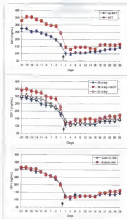


Figure 4-2: Least square mean concentrations of YOP-1 in plasma during the transition period (-25 d through 30 d). Arrow indicates timing.

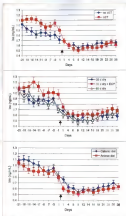


Figure 8.3 Least squares mean concentrations of PKB in plasma during the treatment period (-21 d through 28 d). Arrows indicate dosing.

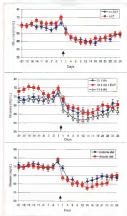


Figure 1-4 Time course of plasma concentrations of glucose in plasma during the intensive period (-21 d through 28 d). Arrow indicates cabing.

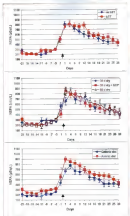


Figure 4-5. Leafy vegetable tissue concentrations of NHPA in plants during the transition period (10 d through 30 d). Arrows indicate starting

does (7.29 ng/mL vs 6.42 ng/mL, respectively). Although plasma concentrations of NEFA did not differ between cows fed the anionic (274.9 ng/mL) or cationic diet (279.7 ng/mL) during the prepartum period, plasma concentrations decreased significantly from d -31 to d -1 for both groups (Figure 4-2). However, there were no differences in the final concentrations between treatment groups achieved before calving (204 vs 208 ng/mL, respectively). When concentrations of BGLU also were tested to be greater on d -31 for cows on both groups, concentrations of BGLU in plasma declined as they approached calving in cows fed anionic or cationic diets (Figure 4-3). However, cows in anionic diet group tended to have statistically greater (not significant) mean concentrations of BGLU than cows fed cationic diet during the overall prepartum period (1.00 vs 0.91 ng/mL, respectively; Table 4-10).

Mean concentrations in plasma of urea did decrease from pre to Table 4-10. When glucose concentrations of cows fed each diet in diet fed prepartum (70.7 vs 76.2 ng/mL). However, mean concentrations of NEFA in plasma were greater (41%) for cows fed cationic diet (268.4 μ Eq/L) than cows fed anionic diet (228.2 μ Eq/L), although concentrations of NEFA increased sharply in both groups of cows during the last days of the prepartum period (Figure 4-3).

Postlactation period

Another objective of the current study was to evaluate the metabolic response of cows during the early postpartum period (days 1 to 21 d) which included 21 d of the postpartum phase of the transition period. To accomplish this, a second series of analyses was performed to evaluate data collected during this postpartum time period.

No differences were detected for mean concentrations of DGL, glucose or NEFA or trends in concentrations due to MIT treatment during the overall postpartum period (d 1 to 28) (Tables 4-8 and Figures 4-2 through 4-5). On the other hand, treatment differences were detected for plasma concentrations of ST and KGF-1. Least squares analyses of variance are in Tables 4-11 and 4-12. Results showed significant effects of MIT for both ST ($P < 0.004$) and KGF-1 ($P < 0.001$) during the 28 d postpartum period. No differences among the three dry-period groups were detected for mean concentrations of ST, KGF-1, DGL, glucose or NEFA during the overall postpartum period. No significant postpartum effect of postpartum day was detected during the postpartum period for concentrations of ST, KGF-1, DGL, glucose or NEFA. However, there were significant two-factor interactions MIT*DAY for DGL ($P < 0.003$) and NEFA ($P < 0.004$), and MIT*DAY ($P < 0.044$) for KGF-1.

Mean plasma concentrations of ST during the early postpartum period (days 1 to 28) are in Table 4-8. Mean concentration of ST during the first 4 wk postpartum period differed due to treatment ($P < 0.001$). Cows in nonparturient control group had lower mean concentrations of ST in plasma (3.12 ng/mL) than MIT treated cows (16.13 ng/mL). The cows treated with MIT had greater mean concentrations of ST (44%) and they remained greater throughout the early postpartum period than nonparturient cows during the same time period (Figures 4-4). Concentrations of ST in control cows remained essentially constant throughout the 28 d period.

Least squares mean and SE of KGF-1 concentrations during the overall postpartum period are in Table 4-9. Mean concentrations of KGF-1 during the overall postpartum period also were greater concentrations for the MIT treated group (150.2 vs 17.74 ng/mL).

and they were maintained at greater than or equal to zero throughout the early postpartum period (17–19%). Cows in both treatment groups had greater plasma concentrations of IGF-I prepartum, and there was a continuous decrease up to parturition with slightly lower plasma concentrations observed during the first 2 wk following parturition (Figure 4-7).

During the early postpartum period, mean concentrations of IGS in plasma did not differ between hMT treatments (9.62 vs 9.24 ng/mL). Plasma concentrations tended to decline further during the first week following parturition and a slight lower trend was observed at concentrations during the remainder of the 28-d postpartum period (Figure 4-8). Concentrations of glucose in plasma followed the same trend as IGS and no differences were observed in mean plasma concentrations after calving (91.3 vs 92.1 mg/dL). Mean concentrations decreased following calving for the first 2 weeks and then increased slightly during the last 2 wk of the 28-d sampling period (Figure 4-9). However, postpartum concentrations of glucose in plasma were significantly lower than prepartum concentrations.

Mean concentrations of NEFA during the early postpartum period are in Table 4-6. Plasma concentrations of NEFA in both groups followed the same trend and no differences were observed due to hMT treatment (138.2 vs 134.2 $\mu\text{Eq/L}$). Concentrations were greatest around time of calving and remained greater for both hMT groups and increased over time during the first 2 wk following calving, but decreased continuously through the 28-d sampling period (Figure 4-5).

Mean plasma concentrations of IGT , IGP , IGF-I , IGS , glucose and NEFA did not differ during the 28-d postpartum sampling period among the three-day period treatments. Mean

plasma concentrations of ST were least for cows in 30-d dry without ECP (7.8 ng/mL), whereas cows in 30 d dry + ECP and 60 d dry groups had similar and numerically (but not significantly) greater mean concentrations of ST (8.4 and 9.3 ng/mL , respectively). Mean plasma concentrations of ECP II were greatest for the cows in 30 d dry + ECP group (140.7 ng/mL), whereas cows in 30 d dry and 60 d dry groups had lowest mean concentrations (121.2 and 121.3 ng/mL). Concentrations were least around parturition and remained low during the 28-d postpartum period (Figure 4-2).

Mean mean concentrations of INS and glucose differed during the first 28-d postpartum differed due to dry period treatment (Table 4-3). Cows in 30 d dry + ECP group had greatest mean concentrations of INS (5.45 ng/mL) and glucose (83.6 ng/dL) in plasma, whereas cows in 30-d dry group had intermediate concentrations of INS (5.39 ng/dL) and glucose (82.7 ng/dL) in plasma, and cows in 60 d dry group had the lowest concentrations of both INS (5.34 ng/dL) and glucose (81.5 ng/dL) in plasma. Plasma concentrations of both INS and glucose tended to decrease during the first 3 wk following parturition with a slight increase observed during the following week for glucose (Figure 4-3 and 4-4). Decreases in concentrations of glucose in plasma of 60 d dry cows was most pronounced but did not differ significantly from the other two groups (Figure 4-4). Mean concentrations of NEFA in plasma did not differ among dry period treatments during the early postpartum period. Plasma concentrations of NEFA were greatest around calving but decreased for the three dry treatments afterwards (Figure 4-5).

Mean concentrations of ST during early postpartum period did not differ due to the dry period treatments. Cows fed the control diet had slightly greater mean concentrations of ST than cows fed the oatmeal diet (9.13 ng/mL and 7.10 ng/mL , respectively). Plasma

concentrations of KGE-1 during early postpartum period were about 80% of prepartum concentrations and did not differ between cows fed acetate (3.26 \pm 1.49 ng/ml.) or untreated diets (3.61 \pm 1.49 ng/ml.) during the postpartum period. Mean concentrations of BQ also were less ($P < 0.05$) during early postpartum period than during prepartum period (Figure 4-2), although concentrations were considered slightly, but not significantly, greater for cows fed acetate diet (0.43 vs 0.57 ng/ml.).

Mean concentrations of glucose for cows fed the two diets are in Table 4-13. Mean plasma concentrations of glucose did not differ for cows fed acetate or untreated diets. However, cows on acetate diet propensities to have slightly greater mean concentrations of glucose than cows fed untreated diet during the early postpartum period (32.4 vs 30.9 mg/dL) about 12 and 15% less respectively than concentrations during prepartum period. Plasma concentrations of NEFA followed the same pattern as cows fed the two diets during the postpartum period (Figure 4-3). Plasma concentrations of NEFA were greater, varied widely, but decreased markedly during the 30 d postpartum sampling period, although mean concentrations still were about double the mean prepartum concentrations (3.21 to 1.1).

Milk, 3.5% PCM and BCM Yields

Least squares analyses of variance for MY for the first 21 wk and milk, 3.5% PCM, and BCM yields during the first 18 wk are in Tables 4-11 and 4-14, respectively. A trend toward significant difference in milk, 3.5% PCM and BCM was detected during LMT treatment during first 18-wk ($P = 0.074$, $P = 0.093$ and $P = 0.023$), respectively, and for first 21 wk for MY ($P = 0.057$). Untreated cows had significantly greater BCM during the first 18 wk ($P = 0.046$, Table 4-15). No significant differences were detected for dry

period and proportion-diet treatments for milk, 1.8-1% PCM, or BCM yields during first 30 wk period. Mean daily yields of milk for dry period and proportion-diet treatments did not differ during overall lactation period (1 to 31 wk).

Results indicated that cows treated with MT had greater mean milk, 1.2-1%PCM, and BCM yields (26.6 kg/d, 42.1 kg/d and 40.9 kg/d, respectively) than control cows (26.7 kg/d, 36.9 kg/d and 37.3 kg/d, respectively) during first 30 wk (Table 4-13). No differences were observed as percentages of protein (1.46 vs 1.87%) or fat (3.33% vs 3.54-3%) between MT treatments. However, cows in unsupplemented control group had significantly greater BCC than cows treated with MT during the first 30 wk of lactation (327 vs. 300x10³). Milk yields during the first 31 wk also were greater for MT treated than cows from unsupplemented (40.1 vs. 37.6 kg/d, respectively; Figure 4-4).

To evaluate the trends in mean MTY during the first 31 wk postpartum, regression curves also were plotted using coefficients obtained from cubic regression analysis for MT treated and unsupplemented cows (McLaren et al., 1992). Test of homogeneity indicated there was no evidence to indicate that curves were not parallel. Cubic order regression curves for milk yield (Figure 4-7) indicated cows treated with MT prepartum and postpartum had greater and more sustained increase in milk production compared to cows in control group that were unsupplemented and daily yield continued greater during the overall postpartum period even though both groups were injected with full dose of MT beginning 45rd day postpartum.

Least squares means for milk, 1-1%PCM and BCM yields for dry period treatments during first 40 wk are in Table 4-16. No significant differences among dry period treatments were detected for any measure of MT evaluated. Cows in 0.04 dry

Table 4-13 Least Squares Analysis of Variance for Milk Yields of Holstein Cows During gestation period (1-21 wk)

Source	df	SS	F	P<F
MC ¹	1	4075.08	1.14	0.0079
SEA ²	1	1607.26	1.46	0.2021
DET ³	1	31.71	0.04	0.8908
DET ⁴	1	186.47	0.12	0.7279
MC*DET	1	6684.12	1.20	0.2062
MC*SEA	1	114.75	0.09	0.7628
MC*DET	1	39.11	0.02	0.8774
SEA*DET	1	1428.91	1.18	0.2950
DET*DET	1	492.45	0.29	0.5839
DET*SEA	1	236.09	0.23	0.6302
MC*DET*SEA	1	1847.97	1.44	0.2446
MC*SEA*DET	1	1760.79	1.77	0.1864
DET*SEA*DET	1	461.62	0.74	0.3911
MC*SEA*DET	1	1.92	0.00	0.9790
MC*SEA*DET*DET	1	1758.58	1.41	0.2411
Cov*(MC*DET*DET*SEA)	49	1547.58	435.11	0.0001
WE ⁵	1	434.38	11.97	0.0004
WE*WE	1	26324.42	6234.49	0.0004
WE*MC*WE	1	11246.43	454.60	0.0004
Error	1407	26.11		

MC¹=Milk yield during lactation (1-14 wk) (Source: 1=61.1 kg MC/L), ²SEA=Source (1= cows with dry periods during last months (September/October, March, April and May), 2= cows with dry periods during cold months (November/December, January, and February), 3=1st dry period treatment (1= 20-1 dry period, 2= 3rd dry period), 4= 5th dry period), ⁵WE=Type of farm of origin (1=Farm of origin 1, 2=Farm of origin 2), WE*WE=Type of farm of origin for WE cows, where are Type 1).

Table 8.10. Least Square Means and SE of Milk Yield, 3.5 % FCM and SCC of Holstein Cows During Early Lactation

Measurements	Lact Treatments					
	I			II		
	Mean	SE	DF	Mean	SE	DF
Milk Yield (kg/d) ^a	34.7	0.1	427	39.4	0.1	429
3.5% FCM (kg/d) ^a	34.9	0.1	426	42.1	0.1	427
SOM (kg/d) ^a	21.5	0.1	426	48.5	0.1	426
Milk Yield (kg/d) ^a	21.0	0.1	429	48.1	0.1	426
30d Milk Yield (adjusted, kg) ^b	1012	0.156	10079	10079	0.156	10079
Peak (ML Milk Yield (kg) ^c	10403	0.154	10549	10549	0.153	10549
Current ML Milk Yield (kg) ^c	1438	0.188	10079	10079	0.187	10079
Apparent Efficiency ^d	1.08	0.001	426	1.20	0.001	426
Scale Efficiency (kg/Meat) ^e	0.04	0.001	426	0.27	0.001	426
SCC ^f 1-4	627	0.194	529	529	0.194	529
% Protein ^g	2.86	0.01	426	2.87	0.01	426
% Fat ^h	3.93	0.05	529	3.96	0.05	529

^aTreatments I-10 vs. lact. Treatment II-10 2 kg 3.5% FCM through 30d of postpartum.

^bDuring 1-10 wk postpartum.

^cDuring 1-31 wk postpartum.

^dAdjusted for previous lactual 30d of milk yield.

^e30-100 Meats: lactation milk yield.

^fDuring 1-4 wk postpartum.

^gSCC: Somatic Cell Count $\times 1000$.

^hW-0.1, W-0.02, W-0.01, W-0.05.

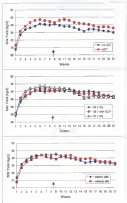


Figure 4-3: PK/PD production of Meloxicam versus hours during early isolation. Arrow indicates the time full dose of NST regimen started

treatment did have slightly greater numerical milk (30.4 kg/d) $\pm 1\%$ PCM (41.6 kg/d) and SCC (21.5 kg/d) peaks than cows on 30 d dry with no DCP (27.6 kg/d , 39.3 kg/d and 21.9 kg/d , respectively) and then cows on 30 d dry + DCP (37.9 kg/d , 48.7 kg/d and 24.1 kg/d , respectively), but, as indicated, they did not differ significantly. Furthermore, MY during first 20 wk did not differ significantly due to dry period length or treatment (Table 4-6). No significant differences in percentages of positive (2.78 , 1.93 and 2.49% , for <3.00 , 4.00 and $>4.04\%$) or SCC (477 , 495 and 509×10^3) were detected due to proportion dry treatments (30 d dry, 30 d dry + DCP and 30 d dry, respectively) during the first 30-wk lactation period (Table 4-6).

Cubic spline regression curves were calculated for MY to describe the trend trends for the individual treatments over the 21 wk lactation period (Figure 4-7). Tests of heterogeneity detected evidence that curves were not parallel ($P < 0.01$) (Nelson et al., 1995). Cows on 30 d dry had highest milk yield that were greater at the beginning of lactation but had lowest milk yield after 18 wk. Cows on 30 d dry with and without DCP had similar milk yields after parturition.

No differences were observed in mean milk, $\pm 1\%$ PCM, and SCC peaks and percentages of positive, or fat or SCC, for the cows fed different proportions diets (protein or vitamin) during the first 30-wk period. Similarly, mean MY during the first 21 wk did not differ significantly due to proportion diet fed the cows. Milk yield of cows fed various groups were about 1 kg greater during first 18 wk (34.7 vs. 32.5 kg/d). However, over the first 21 wk period, the small numerical difference disappeared and mean daily MY were similar for the two proportion diet treatments (33.6 vs. 33.3 kg/d) (Table 4-13).

Table 4.10 Least Square Means and SE of Milk Yield, Lactation PGM and SCC of Holstein Cows During Early Lactation/Postpartum in Clinical State During Pregnancy/Post

Measurements	Pregnancy Day Treatments ^a					
	I			II		
Milk Yield (kg/d) ^b	38.7	±	0.28	37.3	±	0.29
Lactation PGM (kg/L) ^c	48.9	±	0.33	49.2	±	0.36
SCM (kg/d) ^d	39.4	±	0.32	38.6	±	0.35
Milk Yield (kg/L) ^e	38.8	±	0.31	38.3	±	0.33
100d Milk Yield (adjusted) (kg) ^f	9793	±	283	9998	±	283
Pregnant/ML Milk Yield (kg) ^g	10044	±	239	10247	±	279
Current ML Milk Yield (kg) ^h	9793	±	290	9995	±	291
Apparent Efficiency ⁱ	1.09	±	0.02	1.09	±	0.02
Gross Efficiency (kg/Min) ^j	0.70	±	0.04	0.72	±	0.04
SCC (x10 ³)	42.9	±	40.6	42.1	±	40.9
N/Pregnant ^k	2.84	±	0.04	2.89	±	0.04
N/Post ^l	2.82	±	0.05	2.81	±	0.05

^aTreatment I=Anovus and pregnancy, Treatment II=Calvege and pregnancy

^bDuring 1-18 wk postpartum.

^cDuring 1-10 wk postpartum.

^dAdjusted for previous lactation 100 d of milk yield.

^e100d of lactation equivalent milk yield.

^fDuring 1-4 wk postpartum.

^gSCC=Sommer Cell Count of 1000

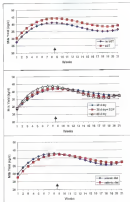


Figure 4.7 Cubic regressions depicting changes in weekly milk yield of Holstein cows during the experiment. Arrow indicates the time full dose of HST injection started

Using coefficients obtained from cubic regression analysis, the trends in serum MT were evaluated during the first 21 wk postpartum period for preparation groups and lactation data (Wilson et al., 1998). For preparation data, there was no evidence detected to indicate that individual MT curves were suspended during the early postpartum period (21 wk), as determined by heterogeneity test (Figure 4-7). Although MT of cows fed oatmeal diet initially showed greater daily production, decline in MT also started earlier than that of cows fed oatmeal diet and overall result was that there was no change in mean MT during the first 21 wk in lactation.

Apparent efficiency (MT/kg/305-day) and gross efficiency (MT/kg/MEI/Mod) of milk production were calculated for all treatment groups (Tables 4-15, 4-16, 4-17). Apparent efficiency of milk production was greater for cows injected with MT than for control cows (1.31 vs. 1.16, $P < 0.05$). No differences in apparent efficiency of milk production were detected among dry period treatment groups or for the preparation diet treatment groups (Tables 4-16 and 4-17). Gross efficiency of milk production followed the same trend. Gross efficiency of milk production by cows injected with MT was significantly greater, therefore these cows were more efficient than cows not injected with MT (0.17 kg/MEI vs. 0.16 kg/MEI, respectively; $P < 0.05$, Table 4-16). Gross efficiencies were 0.16, 0.16 and 0.17 kg/MEI for cows in 30 d dry, 30 d dry + ECP and 60 d dry groups, respectively ($P > 0.24$, Table 4-16). No difference in gross efficiency of milk production was detected for cows fed preparation oatmeal (0.19 kg/MEI) or oatmeal diet (0.17 kg/MEI, Table 4-17).

The percow lactation 305 d intake equivalent (MEI) MT of cows did not differ significantly in MT and non-MT groups (10420 \pm 104 vs 10440 \pm 215 kg, respectively).

Table 4-13). Overall 30-d dry) 1074 ± 103 kg, 30-d dry + ECP (1060 ± 120 kg) vs 60-d dry groups (1070 ± 142 kg, Table 4-13) had similar previous lactations, 1091-d ME milk yields. The previous 305-d ME milk production of cows fed aniseed or ruminant diets prior to this study also did not differ significantly (1064 ± 103 vs 1042 ± 115 kg respectively, Table 4-13). The current lactation 305-d ME milk yields of cows also were analyzed. The current 305-d ME milk yields of cows injected with BST (1073 ± 111 kg) was significantly greater than cows not injected with BST (943 ± 104 kg, $P < 0.01$, Table 4-13). The current 305-d ME milk yields 1068 ± 142, 913 ± 113 and 930 ± 103 kg for cows in 30-d dry, 30-d dry + ECP and 60-d dry groups, respectively (Table 4-14). No differences in current 305-d ME milk production were detected for cows fed preparations aniseed (1110 ± 134 kg) or ruminant diets (910 ± 111 kg, Table 4-14). The current 305-d ME milk yields also were adjusted for actual 305-d MEY in the lactation that preceded the experimental dry period. The adjusted 305-d ME milk production was significantly greater for BST injected (1007 ± 106 kg) than non-BST injected cows (942 ± 106 kg, $P < 1$, Table 4-15). No significant differences in the adjusted 305-d ME milk production of cows assigned to 30-d dry, 30-d dry + ECP and 60-d dry groups were detected (1000 ± 111, 904 ± 106 and 916 ± 110 kg, respectively, Table 4-16). The adjusted 305-d ME milk production of cows fed preparations aniseed or ruminant diets also did not differ significantly (1000 ± 104 vs 990 ± 104 kg, Table 4-17).

Discussion

Economics, Growth Factors and Metabolism

A series of physiological changes must occur for cows to have a healthy transition into lactation and satisfactory milk production throughout the lactation. It was

hypothesized that SST injections prepartum and postpartum would have a primary effect on directing the use of absorbed nutrients as all directed by altering the metabolism of different tissue types directly (e.g., liver, adipose) and indirectly (e.g., mammary gland) mediated in large part by IGF-I (Bauman, 1992). Somatotropin decreases sensitivity of IGF receptors to IGF in the peripheral tissues and decreases overall uptake of glucose in peripheral body tissues. Thus, this maintains the oxidation of glucose to CO_2 in these peripheral tissues and more glucose is available for milk synthesis. Somatotropin also increases lipolysis in adipose tissue. Because glucose is used as the primary energy metabolite and as a substrate for synthesizing milk constituents in mammary tissue, energy needed by other peripheral body tissues would be derived from products of lipolysis or metabolism of non-glucoseorganic compounds arising from the liver and from lipolysis itself. Thus, more glucose would be available to the mammary gland. During negative energy balance, ST also will spare protein the net source of energy in tissues because it increases lipid mobilization and inhibits glucose oxidation in the whole body. Proteins that are mobilized from the muscle can be used in the liver, in the gut, and in the blood which will increase overall metabolism and efficiency of protein use. Adjustments of these endocrinological changes would be especially important during the early lactation because of lower DMI at this time.

Both prepartum and postpartum injections of SST did result in elevated concentrations of ST in the peripheral circulation of treated cows. Concentrations of ST tended to be greater during the prepartum period than during the postpartum period. Although ST concentrations increased around parturition, no net increase in plasma concentrations of ST was observed in untreated cows as they moved from the prepartum

in the postpartum period. Generally, the levels of this study agreed with others (de Boer et al., 1983; Kaprechiev and Trifirov, 1973; Yano et al., 1994). Somatomedin is a somatotrophic hormone that is involved in the regulation of the metabolic adaptations that occur during the transition from the nonlactating to the lactating state. Hence, ST concentrations begin to increase during late pregnancy and rise during early lactation and then fall during the latter stages as IGF declines (Collier et al., 1984). Through these changes, ST can exert a powerful galactopoietic influence once lactation is established, it decreases rates of lipogenesis and activation of lipogenic enzymes and promotes glucose utilization for milk synthesis (Rasmussen and Yano, 1993).

Changes in concentrations of ST following exposure of lactating cows to a wide range of exposure-doses and times within the lactation cycle. Simonson et al. (1998) reported elevated concentrations of ST in plasma of cows whose prepartum exposure of 3 and 14 mg hMT/d were administered. It also was shown that cows that were exposed with 20 mg hMT/d had higher concentrations of ST than untreated cows (Bachman et al., 1992). Lutz et al. (1993) reported an increase in plasma concentrations of ST following exposure of cows with hMT during both mid and late lactation. Additionally, concentrations of ST were elevated throughout the entire lactation period when cows were exposed during both prepartum and postpartum periods (Garcia et al., 2008; Galley et al., 2000). The amount chosen (14.2 mg hMT/d) to inject during the current studies was based on previous studies (Galley, 1994) that indicated the dose was the least of the amount evaluated that brought about desired effects on ST and IGF-I. Thus, increases in plasma ST concentrations in current studies were expected and increase in ST

concentrations were appropriate for the amount of hGH ingested either prepartum or postpartum.

No differences were observed in concentrations of ST in the plasma of cows during the prepartum or postpartum periods in any of the three dry period groups or in groups fed the prepartum amount and various diets. Consequently, no differences were detected in the milk yields of cows due to prepartum diet fed. Increases in concentrations of ST in plasma was not limited to only a type of diet or dry period length, rather the effect was more general. Thus, positive feed of hGH treatment may be expected to work under a wide variety of management strategies. Concentrations of ST during the lactation period were reported to follow the same pattern in a number of published studies. The concentrations of ST during prepartum period were less than postpartum period and ingesting hGH did not change this. However, ST concentrations were elevated during early lactation (i.e. Boar et al., 1986; Kopecká et al., 1973; Válek et al., 1983). Normal physiological concentrations of ST in cows are elevated after parturition, most likely, to facilitate the important changes occurring during early lactation (Dexter et al., 1988).

As mentioned previously ST has a very important role in a homeostatic regulation of metabolism. Typically concentrations of ST in cows begin to increase during late pregnancy and are elevated in milking. Moreover, the glucocorticoid response to exogenous injections of ST during lactation suggests that ST has an important role in many adaptations that occur during the lactation period and that increased concentrations of ST due to hGH ingestion likely enhance these adaptations. Physiological processes such as increase in hepatic rates of gluconeogenesis, reduction in muscle uptake and oxidation of glucose, regulation of adipose tissue, and increase in lipolysis and increased uptake of

substrates used for milk synthesis by mammary gland are derived as metabolic products including liver, adipose, muscle, kidney, intestine and mammary. As evidenced, these changes likely are affected by ST treatments and also because of increases in endogenous synthesis and secretion of ST (Blaumen, 1992). Increased concentrations of ST also decreases the ability of IGF in uterine glycometabolism, inhibits release of DAG, second messenger, and decreases uptake of DAG into the cell. These and other decreases in mammary and extra-mammary metabolism are especially important during the early postpartum period because milk production is initiated and amount of milk produced increases rapidly but DAG is not sufficient to support this process. In the current study, low dose of BST injections before and after parturition increased concentrations of ST and it is likely this rate had many beneficial effects because of the likelihood in concentrations of ST and IGF and decrease in DAG and then lowered increased IGF.

In general, BST injected cows had higher IGF-1 concentrations than control cows during prepartum and postpartum periods of this study, although increase in IGF-1 concentrations after parturition were less than the increase that occurred during the prepartum period (+30% vs +20%). In addition, cows in all three dry period treatments followed the same trend with high plasma concentrations of IGF-1 during prepartum period and a consistent decrease through postpartum with the lowest plasma concentrations observed during the first 3 wk after parturition. These results indicated a relationship between the decrease in energy and specific nutrient intake because of the decrease in DAG second-messenger and decreased concentrations of IGF-1 during this time. Adverse effects of negative energy balance and low plasma concentrations of IGF-1 were reported previously (Rieger et al., 1991; Blaumen et al., 1992; Vorum et al., 1992).

also reported that, when MST was repeated during late lactation, dry period and early lactation, increase in concentrations of KGF-1 were greater during the late lactation and the dry period (when DMI was greatest), than during early lactation when DMI is lowest.

Although plasma concentrations of KGF-1 are under control of SE, nutritional status plays an important role in the regulation of the neuroendocrine axis (Brett et al. 1986). When a diet was fed to steers for a 2 wk period that resulted in severe energy deficiency, the KGF-1 response to MST declined markedly (Elsasser et al., 1989). Cows in early lactation typically are in NER and they have greater circulating concentrations of ST but low basal concentrations of KGF-1. Synthesis and secretion of KGF-1 in response to ST is affected by the energy balance (Phillips et al., 1990) and the ST/KGF-1 axis is attenuated by nutritional status (Elsasser and Vernon, 1993). Restriction of DMI at growing stage decreased the basal concentration of KGF-1 in blood and attenuated the positive response of KGF-1 to exogenous MST treatment (Brett et al., 1989). Moreover, the KGF-1 response to MST treatments was greatest when cows were fed high protein/high energy diets (McGowan et al., 1992). Similarly, in the current study the KGF-1 response to MST was less when pretreatment than it was during the prepartum period.

Nutritional status also regulates the concentrations of KGFBP and changes in concentrations of KGF-1. Vance et al. (1991) suggested that these changes were the result of a change in specific circulating binding proteins. About 75% of KGF-1 are bound bound to KGFBP-1 and circulating concentrations of KGFBP-1 are positively correlated with those of ST (Coffack et al., 1982). Administration of ST in lactating cows (Cobbitt et al., 1992; Elsasser (Brett et al., 1989) and pigs (Walton and Edwards, 1989) causes increases

in concentrations of IGFBP-2. Moreover, infusion of IGF-I into normal adults increases IGFBP-2 (Gajjar et al., 1990).

It seems that IGFBP-2 concentrations are higher and IGFBP-3 concentrations are lower during negative nutrient balance (Vince et al., 1991). In contrast to increases in concentrations of IGFBP-2, concentrations of IGFBP-2 decline in circulation following MST operations in sows (McGowan et al., 1990; Cobelli et al., 1991). The IGFBP-2 binds IGFs to various cell lines *in vitro* (Yang et al., 1989). Local concentrations of IGFBP-2 were greatest during early lactation and least during the dry period when sows were in positive BB. Conversely, IGFBP-2 concentrations were least during the dry period when IGF-I was high, and were reduced during MST administration when IGF-I concentrations were elevated (Vince et al., 1991). Thus, lower IGF concentrations during early lactation probably occur because of the NBB observed following parturition (Vince et al., 1991). Although there was a reduced increase in IGF-I during MST administration during early lactation, greater concentrations of IGF-I found *in vivo* in the MST-treated group than in the untreated group did suggest that increased concentrations of ST *in blood* as a result of MST operations, might have caused a shift in proportions of the various IGF-BPs in plasma and changes in the binding of IGFs to these proteins (Amstrong et al., 1989). Although concentrations of IGF-BPs were not measured during the current study, because of greater concentrations of ST, shifts in the proportions of IGF-BPs in plasma and changes in the binding of the IGF-I to these proteins might have occurred resulting in greater circulating concentrations of IGF-I of the MST-treated sows. Because IGF-I serves as a local mediator of mammary epithelial growth and development,

greater concentrations in plasma might have increased the lactational performance of the lactating cows not treated with melengestrol.

Another important hormone that regulates metabolism in cows is IGF-1. Insulin is considered to be an anabolic hormone. It regulates fat deposition and mobilization from adipose tissue. Moreover, IGF-1 causes a decrease in the concentrations of blood glucose and stimulates uptake and utilization of glucose by the liver, muscle, and adipose tissue, as well as stimulating gluconeogenesis and glycogenolysis, which are two main sources of peripheral blood glucose (Jariw et al., 1993). In the current study, concentrations of IGF-1 were high for control and treatment groups during the prepartum period. Greater prepartum concentrations of IGF-1 were expected because of the greater amounts of circulating glucose and the positive energy balance of the cows during late lactation and throughout much of the dry period (Vitalis et al., 1991). On the other hand, cows treated with melT had significantly greater concentrations of IGF-1. As cows approached calving, IGF-1 concentrations typically would be decreasing and would be further reduced after parturition; concentrations would remain lower throughout the lactation sampling period (18-49). Flegal et al. (1992) also reported that IGF-1 was elevated when melT was injected during late lactation and the following dry period, but concentrations declined during early lactation. Erdmann et al. (1992) also reported that plasma concentrations of IGF-1 decreased as cows approached calving. Thus, results of many studies indicate that the changes in IGF-1 observed were the expected physiological response. In dry cows after calving and during early lactation, there is a strong positive relationship between concentration of IGF-1 in plasma and energy balance (Jariw et al., 1994). Increased concentrations of IGF-1 in plasma are associated with positive EB of the cows. This also

would be expected to cause higher concentrations of glucose in blood during the dry period. Decreased concentrations after parturition likely occurred because of the rapid onset of the negative EB during early lactation (Vinson et al., 1991), as lactation demands for energy and precursors for milk synthesis rapidly increase.

In normal pregnancy, plasma concentrations of INS declined, beginning 3 d before parturition. These results agreed with findings that concentrations of INS declined around parturition (Garcia, 1998; Galey, 1996; Mahan et al., 1997), even in hBT repeated cross. Lactation is characterized by low concentrations of INS and a high BT:INS ratio. Decreases in INS receptors and decrease in concentrations of INS following parturition result in a decrease in β -lipoprotein (beta-lipoprotein, 1997). Despite the reduced concentrations of INS and reduced INS receptor numbers in liver and adipose tissue, INS receptor numbers increase in mammary tissue at parturition (Peterson et al., 1996). During late pregnancy, mammary resistance to INS in adipose tissue causes decreased INS response (response) to decreased adipose tissue lipolysis and FFA mobilization (Peterson et al., 1996). Somatotropin has a negative effect on ability of INS to inhibit gluconeogenesis and it also reduces both INS uptake by the cell and INS protein activity that is necessary for the action of INS. The sum of these changes would lead to an increase in glucose production via gluconeogenesis and priority use of glucose by mammary tissue that could occur.

Treatment of cows with hBT during postpartum period stimulates glucose metabolism in cows. Typical response include decreased whole body oxidation of glucose (Calkins et al., 1998), increased hepatic rates of gluconeogenesis (Graham et al., 1998) and decreased glucose response to INS (Graham and Vinson, 1992). In the current

study, mean concentrations of glucose did increase postpartum in MST treated cows. Concentrations of glucose also were greater during the prepartum period, whereas after parturition there was a decline observed in glucose concentrations. Furthermore, cows treated with a much greater dose of MST during prepartum period had significantly higher glucose concentrations (Peterson et al., 1989). Although no prepartum increase in DMG was observed in MST injected compared to untreated groups of cows in the current experiment, decrease in glucose oxidation and a reduction in the rate of glucose in peripheral tissues likely was the reason for the greater concentrations of glucose postpartum in the MST injected group (de Groot et al., 1987). de Groot (1991) also reported that hepatic glucose production from propionate was enhanced during MST treatment probably because the ability of DMG to inhibit liver gluconeogenesis is negatively affected by MST treatment (Sefton et al., 1990). As a result, it was expected that glucose concentrations of MST treated cows would be greater than in controls.

A decrease in rate of glucose by non-muscular tissues increases availability of glucose for muscular activity as a source of energy and milk component synthesis. Increased concentration of glucose also would be expected when greater concentrations of PG were seen, as during MST injections of cows during the prepartum period. Greater availability and higher concentrations of glucose due to MST injections during the dry period without a change in glucose oxidation by the muscular gland may have resulted, either directly or indirectly, in greater concentrations of PG in cows of the same treatment group. Because more glucose was available from synthesis or reduced peripheral utilization (due to reduced peripheral tissue requirement PG), more PG might have been synthesized and secreted to replace blood glucose. On the other hand, decrease

at concentrations of glucose and no change in concentration of IMV during early lactation might have been due to less availability of glucose in peripheral circulation because of the extremely high demands by the mammary gland cells for synthesis of milk lactose. Although MSE expected cows produced significantly more milk during early lactation, these cows still were able to maintain nearly stable concentrations of glucose in plasma after parturition. Moreover, the additional increase in IMV in MSE expected cows did not cause a decline in concentrations of glucose in the circulation compared to control group.

Under most feeding situations, energy is the major limiting nutrient for high producing cows. High yielding dairy cows are in negative energy balance for up to 3 to 4 h with parturition and there is mobilization of body fat to provide the equivalent of about 20% of the energy found in the secreted milk (Przyrembel, 1987). This means more when cows are fed inadequately formulated diets of fibrous. Because of the increase in demand for energy and nutrients after calving, it is important that preparation for the lactation begins prepartum. The maternal tissues, especially during the last trimester of pregnancy, mostly rely on the mobilization of NEFA and ketone bodies because glucose is used largely for respiration metabolism (Bell, 1995). Thus, increased mobilization of NEFA during the late prepartum period is essential. Decreased ability of IMV to promote lipogenesis and to suppress lipolysis results in decreased de novo synthesis of TG and reduction in the re-esterification of fatty acids arising from the TG (Worrest *et al.*, 1993). Reduced IMV of ruminants before calving also is associated with greater occurrence of NEFA mobilization from adipose tissue before calving (Forsberg, 1986). In the current study, low concentrations of NEFA in plasma were detected up to the final week of pregnancy. During the final week of pregnancy a sharp increase in concentrations of

NEFA is plasma-free used for energy in all ruminant groups (Figure 4-5). During the overall postpartum sampling period, concentrations of NEFA declined after parturition but still were more than double the prepartum concentrations. Concentrations of NEFA tended to be higher during the first week following parturition as compared with 8-12 but then concentrations declined, as they did in untreated cows. Typically, lower utilization of glucose and low energy intake results in greater blood concentrations of NEFA and ketosis during late dry period (Peterson et al., 1994). Transition from pregnancy to lactation is characterized by a sharp increase in flow of NEFA into plasma from adipose tissue and concentrations of NEFA in plasma and adipose tissue mobilization are directly correlated (Krause et al., 1988). The reason for the high release of NEFA may be due to increased lipolysis driven by adrenergic stimulation around parturition (Krause, 1991). Furthermore, the metabolic pathways of de novo fatty acid synthesis, plasma TG uptake and fatty acid oxidation, and the activation of the receptor controlling them are greatly reduced during late pregnancy and early lactation periods (McPherson, 1991). In addition, reduced IGF receptors, reduced estrogen or lower activity of IGF proteins on adipocytes also may be responsible for the further increase in plasma concentrations of NEFA during the post partum (McPherson, 1995). In all these actions would lower availability of NEFA for use by peripheral tissues (rather than use of glucose) because glucose is conserved for mammary use during the lactating stage.

Milk, ECM, and SCD Yields

It is important to compare milk yield of cows in commercial dairy herds. One of the purposes of this study was to establish a pattern for the metabolism of cows in order to

increase milk production. Thus, this research was done to study the effects of: i) low dose of MGT, ii) 30-d and 60-d dry periods, and iii) preparation methods and intervals during dryness of any or combinations of these treatments were beneficial for the health and productivity of cows.

MGT treatment and milk yield

Exposure of lactating lactational performance not only in dairy cows but also in sheep, goats, pigs, rats and horses (Jadhav and Jadhav, 1999). However, most research has involved the dairy cow because of its greater importance as a producer of milk and the economical importance of milk. Even though typical MT responses of cows exposed with PMSG/LH or an increase of 25-33 % (4 to 6 days), up to 40% in MT was reported (Jadhav, 1999). In other studies, response to MGT treatments were negligible during early lactation and use of MGT was recommended for the last 50% of the lactation of dairy cows (Jadhav and Jadhav, 1999). A few studies reported on use of a full dose of MGT (PMSG/LH) that started as early as 10 d following parturition. That, when a full dose of MGT was injected into cows when the EB was negative, MT still increased but it did not as a result less in BCS compared to untreated unilaterally managed control cows (Mondal et al., 1996). Furthermore, treating high yielding dairy cows with MGT during early lactation extended the duration/EB and BCS loss by 20-25-d (Mondal et al., 2000). Thus, EBW was more severe when a full dose of MGT (300 mg MGT/14d) was injected into dairy cows during early lactation. However, when Sastawadi et al. (1991) injected cows with 5 mg or 14 mg MGT/d from 14 d postpartum through till CRM, the injected cows produced more PCM than controls and the cows receiving 5 mg MGT/d maintained BCS as well as the untreated controls. In another study a lower dose of MGT

with expected progenies and postpartum (15.2 mg NBT/d) that resulted in increased DMU of cows after parturition (Gibby et al., 2009). Also, there was less decrease in BCS and BW and treated cows also produced numerically greater daily MY and 3.1% PCM than unsupplemented controls (Gibby et al., 2009). Thus, use of different amounts of NBT during prepartum and early postpartum periods appears to have the potential to improve lactation performance during early lactation and the results of this study (Gibby et al. 2009) encouraged use of low dose of NBT during the transition period as an attempt to positively affect metabolism and increase subsequent milk production of transition cows.

In the current study, cows expected to produce 6.2 mg NBT of prepartum and during the early postpartum period that produced 4.6% more milk and 7.6% more 3.2% PCM than unsupplemented females in the control group during the first 70 DIM (Figure 4-4). Treated cows started lactation with higher daily yields of milk which continued throughout the first 70 DIM. Prepartum and/or postpartum changes in circulating concentrations of ET, IGF-1, IGF and glucose were beneficial to the cow during the transition period and during the lactating phase. Somatotropin is a hypothalamic controller affecting maternal target tissues and it shifts the partitioning of nutrients among various tissues. Somatotropin treatment also reduces the tissue requirements for IGF. It also reduces the use of glucose for fat deposition and fatty acid synthesis in adipose tissue to support the increase in milk synthesis at lactating period. Furthermore, IGF stimulates cell proliferation, an effect mediated by IGF-1. As suggested previously, increased concentrations of IGF-1 might have increased mammary cell numbers rather increased cell differentiation during prepartum and/or early lactation periods (Poulsen et al., 1999). Long term exposure with NBT increases voluntary intake of DM and this increase persists during the next several

that MT is supplemented. Although MT injected cows had greater amounts of milk and/or FCM, these cows had the same amount of BW and MCS compared to unsupplemented cows. This suggests that there was more efficient production of MT and greater DMI during the lactation, especially after cows had calve and started to lose negative energy balance. Thus, the changes in concentrations of metabolic hormones, endogenous, and as a consequence of MT injection, had positive effects on DMI, MCS, BW and MT of cows.

Early postpartum treatment of dairy cows (d. 14 after calving) with 3 or 14 mg MTs resulted +4% increase in FCM yields (Jannasch et al., 1992). Ruckstuhl et al. (2002) also reported a 4% increase in MT when cows were injected with 30 IU of MT during 30-d postpartum. In the same trial, milk fat also was elevated by 24 %. (Jannasch et al. (2000) reported over a 12% increase in MT when fall dose of MT (300 mg for every 14 d) was injected from 10 to 150-d postpartum. However, Ruppel et al. (1999) did not see an increase in MT when they injected the lactating Jersey cows during the postpartum period with a fall standard dose of MT (FCMR/40%). Despite the latter results occurred because cows also were used for milk fever induction and plasma-concentrations of ST and IGF-I in treated cows failed to increase more than occurred in unsupplemented control cows. In the current study, all cows received the fall dose of MT at -40 d in lactation, but MT injected cows still had greater daily MT at 150 d. Cows in both injected and control groups showed increased persistency in yield over the sampling period (150 DIM), and were still producing over 30 kg/d milk at this time. However, cows injected with the relatively low prepartum and postpartum dose of MT responded

to the full dose of propionate and propionate itself improves better than cows in the control group because yield of milk for the treated cows will more greater (Figure 4-4)

The mechanism by which KT affects mammary gland function likely involves the IGF-I system. Administration of KT increases circulating concentrations of IGF-I and IGF-BP 2 and these parallel the increase in milk (Herman and Vemon, 1991). One of the effects that is mediated by IGF-I is increased cell proliferation (Bachler and Hensley 1992). Rosenblatt and Steinberger (1988) reported that IGF-I stimulated DNA synthesis in cultured mammary cells obtained from both pregnant and lactating cows. Thus, increased concentrations of IGF-I in KT treated cows during the propionate and propionate periods might have increased mammary cell numbers during propionate and/or early lactation period (Pharasa et al., 1997). As a result, increase in mammary cell numbers would have resulted in greater milk when activity of these cells was further enhanced by the full dose of KT. Even though it followed a lower dose. Furthermore, when DNA increases during later stage of lactation, more nutrient supply would have been available for the cells which would further support milk production.

Results of the current study indicated that cows treated propionate and propionate with KT did have greater mean milk, 1.1 % PCM, and BCM yields than control cows during first 12 wk (Figure 4-4). However, no differences were observed in percentages of protein or fat in the milk. The two treatment groups of cows had essentially the same DM throughout the first 4 wk propionate and no differences in their BW or BCM were detected. Vaughan et al. (1992) also treated GRP propionate in beef heifers to increase increases of ST before parturition and during early lactation. Treated heifers lost more BW and had delayed ovarian activity, whereas no difference was observed in MT. When

cows were exposed with 20–4 mg MCTH during 4–6-d prepartum; no significant differences were detected in MY of control or MCT-treated cows. Unfortunately the cows assigned to MCT had lower MY potential than controls based on the rate and extent of decline in MY after cessation of MCT exposure (de Boer et al., 1994). In another trial, when Holstein cows received 0, 3 or 14 mg MCTH during the last 40 d before parturition, no differences were detected among treatments in DMI during subsequent lactation (Gonzalez et al., 1994). Except for the cows treated with 3 mg/d of MCT during wk 10 of lactation, BW was negative for all cows during the first 70-d of lactation. On the other hand, studies from our laboratory suggested positive metabolic changes that were beneficial to health and performance of the cows exposed with MCT during both prepartum and postpartum periods (Chen et al., 2000; Galay et al., 2000). Chen et al. (2000) reported that prepartum and postpartum exposure of only 3–4 mg of MCTH increased DMI, MY and efficiency of milk production during the first 40 DMI exposures of 15 long MCTH before and after parturition increased DMI of cows following calving allowing treated cows to recover BW and BCS more rapidly during early lactation even though these cows also produced incrementally greater daily MY and 3–5% PCM (Galay et al., 2000). During treatment with MCT, DMI increase typically occurs within 3 to 4 wk and suggests the cows ability to increase MY. In the current study, the relative increase in MY of MCT-treated cows was greater than the increase in DMI compared to untreated cows. Moreover, BW and BCS loss was not affected by MCT treatment. This implies that, first and foremost, there was an increase in feed efficiency especially during the early weeks of the postpartum period in the MCT-treated cows and this was the source of energy and nutrients needed to allow for the increase in milk production.

As mentioned earlier, when studies were conducted that used different doses of hST during prepartum and/or early postpartum period, results differed with respect to metabolic responses. The variable metabolic status and energy trials that evaluated use of hST during either prepartum or postpartum periods may have been due, in part, to differences among the doses: *first*, the cows were fed, or *due to differences in BW and BCS of the cows during the early phase of lactation*. In some studies, hST failed to increase concentrations of ST and/or IGF-I in the peripheral circulation (Bachmann et al., 1992; Rypud et al., 1994). Rypud et al. (1990) did not observe increased MPV of prepartum hST-treated Holstein and Jersey cows possibly because cows also were fed diets to reduce milk fever. Furthermore, single dose of hST during early postpartum period usually maintains the length of lactation curve in NLB, and there is greater loss of BW and BCS—even though an increase in DMG probably occurs (Bachmann et al., 1996; Moshay et al., 2000). If part of the increase in MPV results from greater mobilization of body-tissue reserves, then good BCS (2.5–3.75; Munk et al., 1993) is critical if they are to be reported with hST prepartum and/or postpartum. Clearly, cows reported with hST require good management and adequate nutrition to produce and reproduce well because the increase in DMG and tissue nutrients available to the cow is supported lactation is delayed.

On the other hand, treatment with hST during both prepartum and postpartum periods likely causes metabolic changes, such as increased lipolysis and gluconeogenesis and increased plasma concentrations of ST, IGF¹ and T₃, other products that are beneficial to health and performance of the cows (Gibby et al., 2000). In their study prepartum and postpartum responses of 15–2 mg hST/d increased DMG of cows after

producers, but there was less decrease in BCS and BW. Cows received BW and BCS were rapidly during early lactation which indicated they reached positive EB more quickly and, in part, this allowed expected cows to produce numerically greater dryloads and 7.0% FCM yields. In addition, Gomez et al. (2000) reported that exposure of 1.1 mg of HEDH before and after parturition increased MY and calculated efficiency of milk production during the early lactation period (0-60 d). Hence, low dose of hST during prepartum and postpartum periods might have the potential to increase MY with no negative effects on BW or BCS of expected cows.

Typically, the gross composition of milk is not altered during hST treatment of cows (Boman, 1992; Chelupa and Galligan, 1995). However, the changes in fat percentage seen in response to hST treatments will vary with the energy status of the injected animals. When animals are in HED during periods of nonreproductive seasons, percentage of fat in milk does tend to increase as MY increases. Thus, if use of hST during early lactation increases energy deficit to a greater extent and for a longer duration, it likely would result in increase in fat percentage in milk (Boman and Farmer, 1992). In the current study, no change in milk composition (fat or protein %) was detected up to 70 DPL. After the test period, cows likely would be in positive-energy balance. This fact alone suggests that apparent differences in the energy status of the expected and unexpected cows were not great during the milk sampling period (1-13 wk postpartum) and agree with the fact that there were no changes in mean BW and BCS during the test period. As a result, the low dose of hST treatment that increased MY seemed to be without negative effects on the energy status of the treated cows and implied that DMI increased.

The amount of body fat estimated by BCS is a good indicator of the energy that is available during lactation to support body maintenance and milk production. The relatively abundant supply of energy potentially available to parents for the difference between dietary energy intake and the requirements for body maintenance and milk production (Goebel *et al.*, 1999). Energy intake is known to increase as lactation progresses and thus BCS, as energy arising from tissue mobilization to support milk production will depend upon the starting and ending BCS (PMR, 1999). One unit change in BCS levels from 1, moderate-3, fat-free was estimated to be equivalent to 56 kg (Ott *et al.*, 1991) or 41.5 kg (Sapkota and Pritch, 1983) less body weight change. Similar decrease in BW and BCS and similar recovery of these in MT expected and unexpected cows during the current study suggests that increase in MT in the MT expected cows follows an increase in DM and an increase in efficiency of utilization of ingested nutrients (DMU) as these cows lactate in lactation.

Although fat and protein percentages in milk of MT treated cows did not differ in current study, cows treated propionate and propionate with MT did show lower SCC levels in milk. Increased MT of cows usually is associated with increases in SCC in milk (Whit *et al.*, 1998). Remains cell count is higher in mammary glands due to damage of tight junctions of cells due to inflammation (Korvach *et al.*, 1999). Thus, increases in milk SCC can be used as an indicator of tissue damage. Various types of stress have been explained as causing increases in SCC. However, attempts to experimentally induce stress in individual cows has shown only modest or no effects on SCC. Although SCC of milk from heat stressed cows increases, some of this increase may be due to decreased milk production because of the heat stress (Page *et al.*, 1979; Page *et al.*, 1979).

Streptococcus spp.) counts generally are lowest during the winter and highest during the summer. High temperatures and humidity do not directly cause increases in SCC. Rather, the increase in SCC is due to greater exposure of the udder to pathogens, which results in more severe infections and clinical mastitis during the summer months (Plummer, 1990). In addition, cows milking long ago found heat stress and its lower reduced immunity, resulting in greater SCC and higher rates of clinical mastitis (Wagner et al., 1993). Although it has been demonstrated that there is a slight increase in mastitis in lactating cows treated with MT, this increase is associated primarily with the increased IMF (Peters et al., 1994). Actually, it has been suggested that ST has a potential role in preventing mastitis in ruminants. Increased vascularization of subgingival ST are seen in cows during experimentally induced mastitis (Ghorvashi et al., 1999). Moreover, treatment of cows with MT during 10 consecutive days starting 2 d after experimentally induced E. coli mastitis showed they had better ability to recover from subclinical mastitis than placebo cows, and recovery was more pronounced in the treated cows (Vidalopoulou et al., 1993). However, beneficial effects were limited to prevent mastitis (Ghorvashi et al., 1999), which suggests that ST seems to protect the blood-milk barrier and restore the integrity of the tight junctions in the mammary epithelium of an inflamed mammary gland. This may occur because of positive effects of ST and KGF-1 on the epithelium, induce mRNA, and for epithelial regeneration as observed in rats (Joshi et al., 1997; Barfield et al., 1987). In addition, administration of MT to lactating cows increases absolute leukocyte counts in blood (Crisper et al., 1991). In the current study, SCC of cows treated prepartum and postpartum with a low dose of MT was significantly less even though IMF of the treated cows was greater. This implies there was a positive effect

effect on total LCT in the milk, perhaps because of some unmeasured contribution of effects, as discussed previously.

Dry period treatments and milk yield

Dry period length has been addressed in a few designed trials. The most crucial problem for the evaluation of different dry period lengths is the relationship between days dry and subsequent milk production (Gordon *et al.*, 1974). It is assumed that when a cow is dried off, the loss in the current lactation will be compensated for by greater milk production during the following lactation. However, the periods of parturition and abortion of lactations are extremely important events that are associated with many problems that may result in removal of the individual cow from the herd or in greatly reduced milk production, especially early in the lactation. So, shortest possible dry period that would allow maximum milk production after parturition must be identified and evaluated. Indeed there are only a limited number of studies, experimental and observational, that have been conducted to establish the relationship between maximum days dry and maximum milk yield in the lactation that follows the dry period.

Forty-five to 60-d dry period length has been recommended for use based on the fact that this would maximize production in the following lactation (Crippen *et al.*, 1974; Dettl and Adams, 1962; Klein and Woodward, 1963; Schaeffer and Henderson, 1972). However, the current study did not detect either a benefit or saving for dry period at 60-d. Cows in 30-d dry group (2850 ± 117 kg) and 30-d dry + ECP group (2873 ± 103 kg) produced as much milk as cows in 60-d dry group (2820 ± 144 kg) in 150 DIM. Moreover, 30-d dry groups produced an additional 1570 kg milk during the extended 30-d lactation period before they were dried off. These results agreed with those previously reported

(Buchanan 1982; Schwenk, 2001). In their studies cows dry for 15–34 d dry produced as much milk as their lactations that had 17 d dry period ($p < 10$). The overall milk yields for both short and long dry periods were about 19,20 and 1948 kg at 325 DDM (Schwenk 2002). Moreover, 30-cows produced about 18,154 kg milk following a 32 d dry period, whereas 9-cows with 40 d dry period from the same herd produced 19,551 kg (Schwenk, 2002). These authors indicated that shorter dry periods can be a profitable practice for dairy farmers. However, lactations, a nonlactating period is necessary for optimal milk production during the following lactation. On the other hand, follow up research should be done to study the effect of short dry period on long term health and longevity of these cows.

Earlier recommendations were that dry periods should not be less than 50 d. Klein and Woodward (1962) utilized 11,39 lactation records from Derry Herd Improvement Association (DHIA) to study dry period length. They found that the optimum dry period was 50 d, cows producing >1000 kg of 4% FCM with 12 mo calving interval (CI). They made this recommendation even though average milk production for 40 to 45 and 46 to 49 d dry periods did not differ significantly from the 50 d dry period. Salaswiler and Steinbock (1972) concluded that cows with dry periods of 30–35 d had the highest milk production during the subsequent lactation. Moreover, Ford et al. (1984) reported that cows dry for 40 to 45 d produced significantly more milk (>450 kg) in the subsequent lactation than cows dry for 40 d or less. Effects of days dry on milk yields of first ($n = 1140$), second ($n = 7140$) and third ($n = 102$) lactation Holstein cows from Tennessee and North Carolina were evaluated by Milburn and McDonald (1990). Milk yields for 30–35–40–45 and 50–55 d dry cows were 135, 473 and 792 kg less than the 40–d

dry periods in both lactations and there was little advantage observed for dry periods longer than 60 d. Observational data will be affected by many factors, in addition to dry period length, that are tightly related to subsequent milk production. For example, data from culling records often will not include the reason why a specific cow was dried off earlier than other cows or why cows were dried off later (with dry times) even when lactation spontaneously in the dry periods will dry off cows early because of insufficient milk production. Thus, the reason why cows had shorter dry periods must often cannot be learned from the milk yield records. Cows with short dry periods also may include those cows that calved early due to physiological problems, infection or exposure to heat stress, among others. This would bias the estimated effect of days dry on milk yield in the subsequent lactation because of potential or actual problems during early lactation associated with early calving; this would affect the lactational performance. As a result, cows in second lactation may produce a loss in the milk production records and this may result in insufficient information to adequately estimate the true effects of dry period length.

On the other hand, early experimental studies also recommended a 90 to 100 d dry period. In one study, Evanson (1961) used five pairs of identical first dry cows. One of the pair of identical cows was given at least an 8 wk dry period, whereas other pairmates were milked continuously for two consecutive lactations. Average milk yield of the continuously milked cows in the second and third lactations was 79 and 47% of the control pairs that had a 60-d dry period. In another study, two quarters of each lactating gland of 2 cows were milked continuously while the other two quarters milks the same cow were dried off for ~ 10 d before expected parturition (Smith *et al.* 1963). The

quarters allowed the dry period of 1 to 3 wk produced 40% more milk in the subsequent lactation (Hatch et al., 1967). However, both trials had too few cow numbers and there only showed the trend for a dry period and the dry period length. The only conclusion from many of these studies would be that maximum gland function from a dry period.

Studies using a designed experimental protocol and including larger numbers of cows have been conducted. For example, Coppock et al. (1974) conducted a 42 cow field trial to evaluate the effects of dry period length on later milk production. Cows were assigned to treatments of 30, 35, 40, 45 or 50-d dry periods. Although the cow numbers were high ($n=100$), only 300 cows ($\sim 30\%$) completed the 42 cow study. At the end of 42 mo they concluded that cows averaging less than a 40-d dry period produced 450 to 600 kg less milk in the subsequent lactation compared to cows having dry periods of 40 d or longer. However, dry period lengths were allowed to have wide changes in each group. Therefore, a cow in 30 d dry group could have had a dry period ranging from 20 to 40 d. Importantly, the average length of days dry between cows assigned to 30 and 50 d dry conditions were only 1 d. This could have seriously biased the estimated effect of the dry period length on subsequent milk production, because the actual days dry varied greatly within the individual groups (Coppock et al., 1974).

As described, there is a little doubt that cows need a dry period if they are to reach maximum possible MT that is determined by genetics and management. The exact length of time needed for the dry period has not been established definitively and likely is importantly influenced by the time needed for mammary involution. The time course and degree of mammary involution that occurs in cows differs noticeably from that seen in rodents (Coppock et al., 1977) which makes it difficult to model and evaluate the correct

dry period length for cows by using a negatively responding short lactation period. Inclusion of the mammary gland tissue at a slower rate and slower structure is maintained for a greater portion of the period of involution in dairy cows than in rodents (Capuco and Albers, 1995). Moreover, it has been proposed that the process of mammary involution is completed by 15–40 d into the dry period in dairy cows. A mammary state was achieved at 15 d postpartum for epithelial cells containing secretory vesicles or fat droplets and mammary luminal area decreased to its minimum (Capuco et al., 1997). The finding differs greatly from previously held view accepted of end extent of involution in dairy cows but does support the results obtained during the current study based upon lactation performance. Shorter dry periods did not negatively affect subsequent lactational performance compared to cows given 60 d dry period. Although extended 17% lactations have been suggested as a way to increase rate of mammary involution in cows (Albers et al., 1994), no benefits of ECF regimens were seen in the current study. Also, no significant differences among dry period treatments were detected for any measure of MY evaluated in the current study. This observation suggests that mammary gland involution and remodeling apparently can be completed within ~ 30 d. Thus, 30 d dry period should be long enough to allow cows to produce milk following parturition similar to that of cows that had essentially double the dry period length.

Emulsion diets did not milk yield

Amount or release of milk fat during the prepartum period did not affect MY during the first 21 wk postpartum, or MY or milk components during the first 10 wk postpartum. Moore et al. (2000) indicated that when cows are fed prepartum diets that had DCAI of 15, 30 or 45 mg/100 g of dry matter, no observed effect on MY or any milk constituents

are observed from 2 to 30 wk postpartum. On the other hand, MY increases when low DCAD diets are fed (10.7 vs 10.0 g/100g DM) prepartum compared with high-DCAD diets ($+15$ vs 10.0 g/100g DM) (Went et al., 1993). In contrast, MY was not significantly improved when a negative DCAD diet (-7 vs 10.0 g/100g DM) was offered 3 wk prepartum compared to high DCAD diets ($+30$ and $+55$ vs 10.0 g/100g DM) (Lopez et al., 1997). Furthermore, lactation performance of cows was greater when DCAD was between $+30$ and $+50$ vs 10.0 g/100g dietary DM during lactation (Bendall et al., 1994). In the current experiment, diet treatments affected no differences in DM during prepartum or postpartum period or serum concentrations of Ca. Therefore, it seems that either positive or negative DCAD diets can be fed prepartum to the lactating cows as long as Ca is not at below 1.2% as percent of the dry matter (Goff et al., 1997).

Conclusions

Injection of a low dose of MT (10.3 mg/MT/d) during late prepartum and early postpartum periods (21 to +18 d) caused increased prepartum concentrations of ST, IGF-I, IGF and glucose and also postpartum concentrations of ST and IGF that no change in prepartum concentrations of glucose and NEFA. Treated cows produced more milk (3.3% ECM) and BCM from parturition through 18 wk, and milk through 21 wk. When both treated and untreated cows received a full dose of MT (100 mg/100/14 d) starting about 60 DIM the increase in milk production was maintained longer through 21 wk in the MT cows. The mechanism(s) by which greater MT was correlated by the low dose was not identified but undoubtedly was the result of a complex interplay of hormones, growth factors and metabolites in various organs and tissues, as well as other factors including increased DM. Low dose of MT may have improved efficiency of

mammary cell activity and/or numbers. However, it is likely the effect is more wide spread than just in the mammary gland.

There was no evidence that shortening the dry period to ~30 d caused a reduction in milk production. All cows were at adequate BCS before drying (FFC > 2.0) and producing sufficient amounts of milk (~ 1.5 kg/d). Cows assigned to the three dry period treatments had almost identical total milk yields at the end of the 21 wk observation period. Providing RCP at time of dry \times 0 did not improve milk production of 30-d dry group through 150 d. Based upon milk production responses to ~30-d dry period was sufficient time for the mammary gland to involute, for epithelial cells to differentiate, and for a new lactation to be established.

Progestagens did not affect prepartum or postpartum DMI and subsequent milk production or milk composition. Progestagen sources that treatment did not have a significant effect on plasma concentrations of Ca and the outcome diet was just as effective as the source diet for maintaining plasma concentrations of Ca before and after calving.

CHAPTER 5 GENERAL DISCUSSION

After the termination of pregnancy, initiation of lactation necessitates a high degree of integration between the mammary gland and rest of the body. Lactation creates demands on the body of such a magnitude that the physiology of the mother differs greatly from that of the nonlactating state. The metabolic changes that occur ensure that the mammary gland is supplied with nutrients adequate to sustain an appropriate level of mammary activity. The metabolic requirements are especially demanding in highly selected dairy animals. A Holstein cow yielding 40 kg of milk daily secretes around 2 kg of lactose, 1.4 kg of fat and 1.2 kg of protein. Thus, daily feed intake (DMI) increases by more than 40-50 % in high yielding cows during lactation compared to nonlactating state. However, peak DMI does not occur immediately following parturition and therefore, increased nutrient demand of the lactating mammary gland cannot be met exclusively by increased DMI. High level of milk secretion associated with high demand for glucose, fatty acids and amino acids generally gives rise to increased hepatic gluconeogenesis, and increased mobilization of body fat and protein reserves. In addition, the mobilization of these lactating nutrients (glucose, amino acids and lipid precursors) is reduced in less priority organs to support increased mammary gland activity. All these physiological changes in the metabolism of various organs and tissues ensure the adequate supply of available nutrients to the mammary gland. These modifications in the partitioning of nutrients during lactation are considered to be the consequence of homeostatic control

Metabolic adaptations of organs and tissues are closely regulated by the clearance of responses to hormones controls. During early lactation there is a diminished whole body utilization of glucose and reduced responsiveness of the lipolytic system and NEFA mobilization in IM. Reduced responsiveness to IM with the onset of lactation decreases the ability of IM to inhibit gluconeogenesis in the liver and to stimulate lipogenesis in adipose tissue. In addition, glucose uptake in skeletal muscles and glucose oxidation in the whole body is decreased. Thus, a moderate degree of IM resistance in adipose tissue and liver muscle may permit the mobilization of NEFA, and reserve acids and spare glucose for other priority needs.

Glucocorticoids (cortisol) are mostly responsible for regulating the metabolic adaptations that start during the transition period including partitioning of nutrients to adipose tissue, liver and skeletal muscle. Somatotropin is an important homeostatic hormone. Along with its growth-promoting effects, ST also alters tissue responsiveness to IM and catecholamines. Reduced responsiveness to IM decreases rates of lipogenesis and the activation of key enzymes such as acetyl CoA carboxylase, the rate limiting enzyme in fatty acid synthesis from acetyl or glucose. In addition, ST dramatically attenuates the lipolytic response to catecholamines. High plasma concentrations of ST during late pregnancy may reduce IM receptors on adipocytes, inhibit the action of a membrane receptor or inhibit the IM proteins required for action of IM. Thus, ST has a pivotal role in a homeostatic control on metabolism and nutrient partitioning (carbohydrates, lipids, proteins, and minerals) in the cow during the transition period.

De novo estradiol-17 β (hET) is one of the major products of biotechnology that has been developed and used in commercial dairy farms with a resultant unexpected increase in milk production by dairy cows when injected during an oestrous luteal phase. Obtaining a milk yield response to hET does not require special diets or different feed or products. However, treated cows do require adequate amounts of a balanced diet that contains all nutrients needed to support expected milk production.

Major objectives of an efficient dairy farm operation include a successful lactation, high milk yield relative to the feed costs, reproductive competency and finally the return of the cow to the BCS that existed before lactation so she will be prepared for another lactation. In farm animals a milk yield response to hET treatments has been well studied and fully documented (Barnes, 1989). Milk production response to hET occurs because of its known effects on partitioning of nutrients and because a greater proportion of the nutrient intake is used for milk synthesis. It increases liver glucose output, cardiac output, blood flow to the mammary gland and uptake of nutrients used for milk synthesis by the mammary gland among other effects. In addition, ET decreases the rate of oxidation of amino acid and glucose and glucose clearance. Treatment with hET results in coordinated changes of various organs and tissues which naturally occurs during the transition from a non-lactating to lactating state when circulating concentrations of ET is high. Because of the known effects of hET described above, use of hET during the transition period offers a means to elicit positive and beneficial effects supporting of milk synthesis prior to parturition. These positive effects of hET would allow dry cows to make better transition to lactation when a high demand for nutrient intake occurs. Consequently, injection of a low dose of hET (10.2 mg/d) during the transition period

(-23 d prepartum through +23 d postpartum) and throughout the study lactation period (28 d through 56 d) was considered to have the potential to reduce lactational performance. It was hypothesized that increasing concentrations of MT would augment the metabolic changes that shrink the mammary gland and have a positive effect on DMI.

Data from both the first and second studies suggest that use of 14.2 mg MT/d during late prepartum and early postpartum periods caused no apparent negative effects on the second-crown. Although EA was measured only in the second study during first 4 wk postpartum, NRE was not greater in experimental pairs than in unpaired cows in either study. This observation is rationalized indirectly because the increase in MT and BW and the changes in BCS were equal or better in expected relative to an unpaired-crown. Injection of MT resulted in better recovery of BW and BCS during early lactation, especially after injections were discontinued around 42 d postpartum. During the second study, low doses of MT did not provide a greater loss of BW or a faster rate of decreasing BCS compared to untreated cows. Cows in both groups appeared equally capable of replenishing their body reserves even though all cows started lactation at a full dose (400 mg) around 40 d prepartum, a daily dose that was three times greater than that expected before d 42. Various management treatments did not adversely or positively affect the rate of increase in DMI during the first 23 d postpartum; increase in DMI was equal for both treated and untreated cows. In both studies, MT-treated cows produced more milk and 3.1% PCM during the rejection period. In the first study, no composite effects of MT were detected on MT as evidenced by the fact that the increase in MT did not persist after MT injections were discontinued around 42 d postpartum. This suggested that positive effects on expected milk yield only occurred between prepartum and early postpartum MT.

responses. It is less likely that the amount of tissue in the gland or at least number of epithelial cells in the gland were increased due to the low dose of hMT exposure. Clearly responses should be sustained in order to maintain the synthetic activity of these cells and give further benefits. On the other hand, results obtained during the second study contrast those just described. Treated cows showed a similar increase in milk production through 40 d. However, treated cows also produced more milk during the test period when all cows (controls and treated) were exposed with full dose of hMT (>50 d of lactation). They also had higher concentrations of BT, INS and RGF-1 preparations and higher SE and RGF-1 postpartum. While somewhat contradictory, results of both studies indicate that low-doses of hMT exposed prepartum and postpartum had measurable effects on the mammary gland and very likely on other physiological functions and organs to support the lactation. Although no specific data were collected to show differences apparent as to what these changes were, it is likely that it was the sum of many small physiological changes that occurred and which stimulated greater milk production.

Because hMT of cows in the second study was maintained greater in the prepartum and early postpartum exposed cows whereas hMT response was lost if all hMT was discontinued after 40 d postpartum, this suggests that gland lost the stimulatory action of hMT but likely had potential to respond to a greater level if it had been present. So, while results seem contradictory, they support a general and likely more discrete effect of hMT exposure which results in greater hMT and hMT without apparent negative effects. In a broad view of these actions, hMT perturbed the system in a positive way and made it possible for cows to respond better later in lactation without apparent negative effects on health. Indeed, results indicate the overall effects of prepartum and early postpartum hMT

were beneficial since BW and BCS were better maintained. Changes in concentrations of metabolic hormones, closely coupled with effects on various organs, suggest strongly a beneficial effect of MTE during the treatment period that affected the subsequent milk production whether or not the cows were exposed with the full dose of hST (300 mg hST/14 d) than on milk lactation (mid d vs lactation). Importantly, no adverse treatment effects on calving or proportion of postpartum health status were observed during treatment period for the cows across the treatment. This has been interpreted to indicate that injections of 18.3 mg hST/d can be administered during the prepartum period and probably the postpartum period, to improve metabolic status and improve overall milk yields during early lactation.

In current study, the low dose hST injections (18.3 mg hST/d) also were compared during early lactation period. Paterson et al. (1999) and Badmann et al. (2002) evaluated effects of use of hST during late gestation on subsequent milk production. Although Badmann et al. (2002) failed to detect improved MY for the postpartum hST-treated cows, Paterson et al. (1999) reported a positive subleptotroic response when a full dose hST was injected prepartum/implanting 28 d before expected calving. Gatten (2003) also reached the similar conclusion when a much smaller dose was used. He reported 3.1 mg hST/d before and after parturition, and saw greater hMY response than treating cows only prepartum or only during the postpartum period. One possible explanation for the improved hMY response that is the prepartum hST treatment may be via enhanced IGF-1 production in the treated cows, which, in turn, might have increased overall mammary cell numbers during prepartum injection period and/or early lactation. The potential of hGF-1 is not an anamniotic uterine system (Zickert, 1994) to increase mammary cell numbers and

stores) will differentiate during preparation and early lactation period. Because numbers of secretory cells in the mammary gland is a very important determinant of lactational performance, increased cell numbers would be beneficial. However, there is no conclusive evidence that cell numbers were increased. To fully understand any role IV has upon subsequent/lactile and milk production measures prepartum, less effect of IV treatment effect must be tested alone.

Another way to improve MY, without increasing exposure, would be to increase length of the lactation period. Dry cows require a nonlactating period between successive lactations for optimal milk production during the subsequent lactation. Clearly, the dry period also allows cows to recover body reserves essential to support subsequent lactation. However, body reserves replenished during late lactation seems more efficiently than that replenished during the dry period (Joshi et al., 1997), so cows should maintain or gain BCS before they are dried off. In the current study, although dry period length did not have a significant effect on DMIL, BW or BCS of the cows prepartum, cows provided the short dry period regained more BW and BCS when they went into lactating. In addition, cows given 60-d dry lost more BCS than 30-d dry cows and had less DMIL as open percent BW during the postpartum period.

Although not quantified during the current experiment, incorporating lower dietary changes in the dry cow management program may be beneficial to recover lactation via the maintenance of a desired population of rumen microbes. The large diversity in the types of microbes found in the rumen is a reflection, to some extent, of the nutrients fed. Optimal management and efficient fermentation of feed by the microorganisms depends upon a constant and reliable environment. Changes in

components fed as feed formulations cause a shift in microorganisms in the rumen and also decrease the efficiency of the fermentation and absorption processes. Changing the diet of the animal provides a period of transition as the rumen microbial population shifts and the proportions of the rumen microbial species in the rumen will shift to a new balance, one which best accommodates the dietary change. This is referred to as adaptation of the microbial population. Adaptation may require several days to weeks

In the current experiment, diet changes at 60-d dry-cows (from lucerne diet to FOD diet, from FOD diet to CUD diet and from CUD diet to lucerne diet) likely would have required the rumen and its microbes to adapt three times during a short time period. These changes would probably limit the success in feed intake immediately after parturition. On the other hand, fewer changes in diet of 30-d dry cows might have encouraged maintenance of a more stable rumen microbial population and better rumen papilla development. Thus, it might be advantageous to have fewer diet changes prepartum and to allow cows to replace body condition before they are dried-off. Our results suggested that if an adequate ECS can be achieved before drying-off (> 1.25), there were no advantages, based upon adaptation costs, production, of providing cows with a 60-d dry period compared to a 30-d short dry period. In fact, cows might have better ability to maintain body condition and good health following parturition if shortening of the dry period is coupled with a good nutritional management program.

One of the most important objectives of the dairy producer is to keep cows producing milk as much as possible throughout the year. The change from suckling to lactating stage is very demanding on the cows and results in important metabolic changes and loss of milk production during the time they are not milking. These metabolic

changes may lead to increased incidence of metabolic problems which may affect their health and/or productivity and cause the cows to be removed from the herd. In addition, during the dry period, cows do not produce milk and achieving maximal milk production during the next lactation with the least number of days dry becomes important. Establishing optimum length of the dry period is critical to achieve maximum milk production during the next lactation. Cows given 30-d dry periods yielded essentially the same amount of milk at the end of 21 wk period compared to the industrially managed 60-d dry herdsmen. Similar results also were reported by Buckner (2002) and Schauer (2003). Furthermore, an additional 100 kg of milk was obtained from 30-d dry cows by shortening dry period to only 20-d and milking the cows an additional 20-d. It can be verified that cows with ~20-d dry periods will produce just as much milk as those with 60-70-d dry periods during the next lactation with no other negative effects on the cow as a consequence of reducing dry period length. Thus there is an opportunity of extra milk income being generated for each cow during a lactation. Although the current study did not attempt to quantify economic value of a 30-d dry period, it is possible to roughly estimate potential economic benefits of incorporating a 30-d dry period in dairy management. For example, an additional 12.00 kg (milk) might be expected from the increased days on milk (~20 d), this will milk increase by as much as \$100.00 ($10 \text{ d} \times 10 \text{ kg/d} \times \$0.11/\text{kg}$) per cow. Statewide, 34 million dollars (\$140 MP) 150,000 cows are producing milk in Florida; would otherwise be lost. Furthermore, an additional ~4,500,000 DDM (150,000 cows \times 30 d) would be achieved without the addition of any more cows to the current population. Of course, it would be as to perform the short-dry period practice, cows should be producing adequate amounts of milk at 60-d proportion, when they industrially would be deadweight

They also should have a good ECS (minimum 3.254) and should be provided needed minerals via an excellent feeding program to support their needs. On the other hand, there is an extra feed cost to be accounted for if cows were to have shorter dry periods and it is very unlikely that all cows would qualify for a shorter dry period. Lactation data are relatively easily greater than for dry cows. Thus, income coming from the increased milk yield and the cost of additional feed needs to be determined carefully to achieve economically sound management.

In the current experiment, shortening the dry period did not decrease the yield of milk in the subsequent lactation or during 31 wk postpartum. Moreover, no evidence was detected to suggest that RCP injection at the time of drying off is necessary to achieve maximal milk production during the next lactation. Based upon milk production, it appears that ~ 30-d dry period is sufficient time for the mammary gland to involute and subsequently remodel with differentiation of the epithelial cell population. Clearly, the visible results of the real-time PCR studies (Bachman, 2002; Schauer, 2003) strongly supports need for further research efforts in this area to evaluate potential health effects, any changes in culling intervals, and cow turnover/jeweling costs that may occur. Similarly, it must be determined whether the practice can be used during longer summer months when gestation length may already be shorter and cows would be at risk of too short a dry period if they calved earlier than expected.

On the other hand, most of the cows used in current studies on dry period length had dry periods during the hot summer days. This was purposely selected because of the potential for earlier than expected calving. Most cows during the summer is known to calve early calving. As a result, employing shorter dry periods may result in cows

lactating dry periods less than 20 d. Although BCP experiments did not have positive effects on the lactational performance, there may be benefits of using BCP for cows that would have shorter dry periods, even less than 20 d, because estrogen has been found to increase speed of mammary involution. Administration of estrogen was associated with induction of secretory protein, phosphogluco- and phosphoenolpyruvate carboxykinase in the gland and activation of these secretory proteins are known to accelerate mammary involution (Julian et al., 1993).

Finally, although cow numbers were too few to actually evaluate health status, no apparent health and/or calving problems or benefits were observed for the cows across the dry period treatments. Importantly, no effect of BCP on early calving and/or abortion was observed during the experiment, nor were the cows that calved during the months of September through May, which were somewhat more stressful time periods. Because of the risk of early calving due to heat stress, use of BCP and short dry period (<30) small numbers should be tested during the summer months to evaluate effects on subsequent health and production and if dry periods are actually reduced to less than 20 d.

Hypocalcaemia: is a metabolic disorder of Ca homeostasis that affects dairy cows. This disease is related to the metabolic turnover of Ca. This is especially important for dairy cows around calving because metabolic turnover of Ca is the greatest for coverage of BW at calving and during early lactation. As a result, feeding diets that have a negative DCAI has been recommended and used to prevent cases of milk fever during the last 21 d prepartum and shortly after calving. However, in recent years it has been suggested that high potassium contents of ingredients utilized in close up ration is as important as even more important than modifying blood by feeding negative DCAI diet to prevent hypocalcaemia in dairy cows (Goff et al., 1997). The clinical hypocalcaemia was

observed during that study and the overall serum concentrations of Ca did not differ in cows fed once (30–40 kg/100 kg of ration) or twice daily (10–20 kg/100 kg) before, during, or after calving. Proportion means that treatment did not have a significant effect on plasma concentrations of Ca and the evidence that was just as effective as the ration that for maintaining plasma concentrations of Ca before and during the 30-d immediately after calving. Thus, feeding means that did not result in better maintenance of serum concentrations of Ca in Holstein cows. Additionally, no effects of proportion diets fed were observed on proportion or proportion (DMI, BW or BCB, and feed intake, if any, effect on proportion DMI). In fact, cows fed once and twice daily proportion maintained DMI greater than typically observed during the transition period. The DMI were greater than 21 kg/d if a proportion. High DMI around the calving (the night have helped cows to maintain adequate concentrations of Ca around parturition which, in turn, prevented onset of hypocalcaemia (milk fever) and allowed cows to initiate milk lactation in good health and with greater availability of essential nutrients.

In conclusion, results of these studies suggest that repetition of 50.2 mg of MTG before and after parturition increased plasma concentrations of ST and NCP-1 and a significant increase in milk production during treatment in early lactation. Furthermore, no significant differences in MY of cows provided 30 and 60-d dry periods suggested that a 30-d dry period is sufficient time for mammary gland to mature and milkogenesis. Finally, because no strong adverse effects of MTG or short dry period length were evident, these practices have potential to improve management of transition dairy.

APPENDIX LIST OF SIGNIFICANT TWO-WAY INTERACTIONS

Table A.1 Proportions significant two-way interactions between diet treatments and months for BCS

Treatments				
	DEET PMSA-I	DEET PMSA-II	DEET SPMSA-I	DEET SPMSA-II
Month (n)	DEET PMSA-I	DEET PMSA-II	DEET SPMSA-I	DEET SPMSA-II
DEET PMSA-I	—	NS	P=0.04	NS
DEET PMSA-II	NS	—	NS	NS
DEET SPMSA-I	P=0.04	NS	—	NS
DEET SPMSA-II	NS	NS	NS	—

DEET I=proportion correct diet, DEET II=Proportion correct diet, SEA-I= cows with dry periods during hot months (September–October–March–April–and May's) SEA-II= cows with dry periods during cold months (November–December–January–and February)

Table A.2 Proportions significant two-way interactions between diet treatments and months for BCS

Treatments				
	DEET PMSA-I	DEET PMSA-II	DEET SPMSA-I	DEET SPMSA-II
Month (n)	DEET PMSA-I	DEET PMSA-II	DEET SPMSA-I	DEET SPMSA-II
DEET PMSA-I	—	NS	P=0.1	NS
DEET PMSA-II	NS	—	NS	NS
DEET SPMSA-I	P=0.1	NS	—	NS
DEET SPMSA-II	NS	NS	NS	—

DEET I=proportion correct diet, DEET II=Proportion correct diet, SEA-I= cows with dry periods during hot months (September–October–March–April–and May's) SEA-II= cows with dry periods during cold months (November–December–January–and February)

Table A-3 Post-treatment significant two-way interactions between dry period treatments and season for MIL

	Treatment					
	DWY I PMSA I	DWY I PMSA II	DWY II PMSA I	DWY II PMSA II	DWY III PMSA I	DWY III PMSA II
Interaction	P=0.156	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000
DWY I PMSA I	---	NS	NS	NS	NS	P=0.00
DWY I PMSA II	NS	---	NS	NS	NS	NS
DWY II PMSA I	NS	NS	---	P=0.1	NS	NS
DWY II PMSA II	NS	NS	P=0.1	---	NS	P=0.00
DWY III PMSA I	NS	NS	NS	NS	---	P=0.00
DWY III PMSA II	P=0.00	NS	NS	P=0.00	P=0.00	---

DWY I: MIL diagnosed within 60P; DWY II-III: all dry periods; DWY I: DWY III: 60P dry period; PMSA I: cows with dry periods during hot months (September-October/ March, April, and May); PMSA II: cows with dry periods during cold months (November-December/ January and February)

Table A-4 Post-treatment significant two-way interactions between dam treatments and season for IFP

	Treatment			
	DWY PMSA I	DWY PMSA II	DWY PMSA I	DWY PMSA II
Interaction	P=0.00	P=0.00	P=0.00	P=0.00
DWY PMSA I	---	NS	P=0.00	P=0.00
DWY PMSA II	NS	---	P=0.00	P=0.00
DWY PMSA I	P=0.00	P=0.00	---	P=0.00
DWY PMSA II	P=0.00	P=0.00	NS	---

DWY: Postpartum season; PMSA I: Postpartum season; PMSA II: cows with dry periods during hot months (September-October/ March, April, and May); PMSA II: cows with dry periods during cold months (November-December/ January and February)

Table A.2 Proportion significant two-way interactions between dry period treatment and season for SCC

SCC (log)	Treatment					
	DMY I TREA I	DMY I TREA II	DMY II TREA I	DMY II TREA II	DMY III TREA I	DMY III TREA II
SCC (log)	1.20 (0.00)	0.10 (0.00)	0.10 (0.00)	0.10 (0.00)	0.10 (0.00)	0.10 (0.00)
DMY I TREA I	—	NS	NS	NS	NS	P=0.02
DMY I TREA II	NS	—	NS	NS	NS	P=0.02
DMY II TREA I	NS	NS	—	P=0.02	NS	NS
DMY II TREA II	NS	NS	P=0.02	—	P=0.02	P=0.02
DMY III TREA I	NS	NS	NS	P=0.02	—	NS
DMY III TREA II	P=0.02	P=0.02	NS	P=0.02	NS	—

DMY I = (0-14) days postpartum; DMY II = (15-42) days postpartum; DMY III = (43-60) days postpartum; TREA I = cows with dry periods during last months (September/October/November, April, and May); TREA II = cows with dry periods during cold months (December/January/February and February).

Table A-6 Postpartum significant two-way interactions between ISF and dry period treatments for MS

Lactation	Treatments					
	DAY 1 MS10	DAY 1 MS10a	DAY10 MS10	DAY10 MS10a	DAY 10 MS10	DAY 10 MS10a
Lactation	Pre-Lact	DAY 1 MS10	DAY 1 MS10a	DAY 10 MS10	DAY 10 MS10a	DAY 10 MS10a
DAY 1 MS10	—	NS	NS	NS	NS	NS
DAY 1 MS10a	NS	—	NS	NS	NS	NS
DAY 10 MS10	P=0.01	NS	—	NS10a	P=0.01	NS
DAY 10 MS10a	NS	NS	P=0.01	—	NS	NS
DAY 10 MS10	NS	NS	P=0.01	NS	—	NS
DAY 10 MS10a	NS	NS	NS	NS	NS	—

DAY 1 (first dry period) versus DAY 10 (last dry period) = DAY 10-10-day period, MS10a (seven wet-day periods during last month (September/October/November, April, and May) MS10 (seven wet-day periods during last month (November/December/January and February)

Table A-1 Paired-wise significant two-way interactions between SST and dry-period treatment for H2PA

	Treatment					
	DRY 1 WET 1	DRY 2 WET 2	DRY 3 WET 3	DRY 4 WET 4	DRY 5 WET 5	DRY 6 WET 6
WET 1/2/3/4	WET 1/2/3	WET 2/3/4	WET 3/4/5	WET 4/5/6	WET 5/6/7	WET 6/7/8
DRY 1 WET 1	—	NS	P=0.07	P=0.04	P=0.02	NS
DRY 1 WET 2	NS	—	P=0.03	P=0.11	P=0.04	NS
DRY 2 WET 2	P=0.07	P=0.03	—	P=0.11	P=0.06	NS
DRY 3 WET 3	P=0.06	P=0.04	P=0.03	—	NS	P=0.11
DRY 4 WET 4	P=0.04	P=0.04	P=0.03	NS	—	P=0.04
DRY 5 WET 5	NS	NS	NS	P=0.06	P=0.03	—

DRY 1-10: dry period with no DRY period; WET 1-10: dry period + DRY DRY 10-10: dry period, DRA 1-10: no dry period during hot months (September - October, March, April, and May); DRA 10-10: no dry period during cold months (November - December, January, and February)

Table A.8 Postpartum significant two-way interactions between dry period treatment and season for RFP-L

Treatments						
	DRY I TREA I	DRY I TREA B	DRYII TREA I	DRYII TREA B	DRY III TREA I	DRY III TREA B
DRY I vs TREA I	DRY I vs TREA B	DRY II vs TREA I	DRY II vs TREA B	DRY III vs TREA I	DRY III vs TREA B	
DRY I TREA I	—	P=0.00	NS	P=0.00	P=0.00	NS
DRY I TREA B	P=0.00	—	NS	P=0.01	NS	NS
DRYII TREA I	NS	NS	—	P=0.01	NS	NS
DRYII TREA B	P=0.04	P=0.01	P=0.01	—	NS	P=0.00
DRY III TREA I	P=0.04	NS	NS	NS	—	P=0.00
DRY III TREA B	NS	NS	NS	P=0.00	P=0.00	—

DRY I=0-60 d dry period with no DCP; DRY II=60-90 d dry period + DCP; DRY III=> 90 d dry period; TREA I= P cows milk dry periods during hot months (September - October, March, April, and May); TREA B= cows milk dry periods during cold months (November - December, January and February)

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BIOGRAPHICAL SKETCH

I was born in Antalya, Turkey in 1970. I completed my elementary and middle school education in Antalya and Iskenderun, respectively. Because of the changes in my father's business position, we moved to Eskişehir where I graduated from high school. I sat for a national exam right after my graduation and was admitted into Ankara University, College of Veterinary Medicine. I started my college education there in 1987 and graduated from the University in 1992 as a veterinarian. I worked for 1 year as a veterinarian and then took another nationwide exam for the opportunity to study in the USA for both the Master's and PhD degrees. I spent 4 months at the University of Delaware for English education and started my master's degree program at Clemson University in January 1994. Then, I transferred to the University of Florida, Department of Ovip and Poultry Sciences in August, 1994 to continue to my master's degree and received an MS degree in August 1998. I have been working on my PhD studies since then.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality as a dissertation for the degree of Doctor of Philosophy.


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August 2007


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